ol. 88 Part 2 June 1947

The Quarterly Journal of Microscopical Science

(Third Series, No. 2)

JOINT EDITORS

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Published for the Company of Biologists Limited

OXFORD: AT THE CLARENDON PRESS
LONDON: GEOFFREY CUMBERLEGE

PRINTED IN GREAT BRITAIN
BY CHARLES BATEY, AT THE UNIVERSITY PRESS, OXFORD

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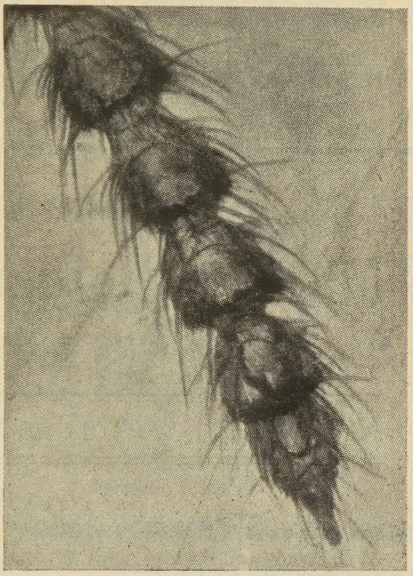

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CAMBRIDGE UNIVERSITY PRESS
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Experimental Data on the Function of the Interstitium of the Gonads: Experiments with Cockerels

BY

J. W. SLUITER AND G. J. VAN OORDT

(*Dept. of Endocrinology, Zoological Institute, University of Utrecht*)

With one Plate and seven Text-figures

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1. INTRODUCTION

THE problem of the localization of the endocrine function of the testes in birds as well as in mammals is still unsolved, notwithstanding the large numbers of papers relating to it. The results of our experiments with young mice, forming the first part of these investigations, have been published by one of us (Sluiter, 1945).

It is generally known that the hypothesis of Bouin and Ancel (1903), who claimed that the testis hormone is formed in the so-called interstitial cells, was violently opposed by Stieve (1921*a*, 1921*b*, 1923, 1926); later investigations, however, especially those of Benoit (1924*a*, 1924*b*, 1929) pointed out that the endocrine function of the cock's testis is not interfered with when the generative tissue is totally eliminated by Röntgen-radiation. Hence we must assume *per exclusionem* that in the avian testis the male hormone is formed somewhere in the intertubular tissue. Benoit (1929) ascribed this function in the cock's testis to intertubular cells, which morphologically resemble gland cells. He identifies them with the well-known so-called Leydig cells, which according to him possess a glandular appearance periodically. Stieve on the contrary does not find any secretory cells in the intertubular tissue. According to him the fully developed Leydig cells, large conspicuous cells containing many lipoid granules, have only a trophic function.

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In the present paper we will try to answer the following questions:

1. Is it possible to locate, in the intertubular tissue of the cock's testis, cells that may be regarded as glandular on the evidence of their cytological structure and the chemical nature of their contents? (Cf. p. 144, under 2.)
2. What is the physiological significance of the intracellular storage of lipoids in the intertubular tissue? (Cf. p. 146 under Conclusion 3.)
3. Are the number, the functional changes of structure, and the microchemical reactions of the contents of the interstitial cells such that these may be regarded as the cells that produce the male sex hormone; and are this number and these structural changes present at an appropriate period of life? (Cf. p. 148 under Conclusion 4.)

We have attempted to answer these questions by a careful cytological investigation of the different intertubular cell-types, and of their changes after the administration of gonadotrophins to cockerels, which manifest their effects by an accelerated development of the head appendages.

2. Material and Methods

The material consisted of 31 cockerels, the ages of which varied between 2 and 200 days. In 5, being 14–52 days old, gestyl, a gonadotrophin prepared from pregnant mare serum, which has a distinct accelerating influence on the development of the head appendages, was administered. Our thanks are due to the Directors of Organon N.V., for providing us with this preparation. Each experimental bird received 4 doses totalling 100 I.U. every other day and was killed for autopsy 2 days after the last injection. Then the testes were fixed and the size of the head appendages measured. Owing to war-time circumstances we did not have the necessary photographic material at our disposal; we therefore had to use translucent paper on which images of the head appendages were projected. To establish the relative surface area of these appendages, these images were redrawn on cardboard, cut out, and weighed.

Parts of the same testis were fixed in Bouin's fluid for general staining; in Champy's fluid for staining cytological details such as mitochondria and granules with the aid of Altmann's acid fuchsin and brilliant-cresylblue; in Giroud's and Leblond's fluid for demonstrating vitamin C; in Schultz's fluid to establish the presence of cholesterol; and finally in formalin for the staining of lipoids with Sudan III. The thickness of the sections was 2–3μ after Champy-fixation and 10μ in all other cases.

3. Growth of the Testis, of its Components, and of the Head Appendages

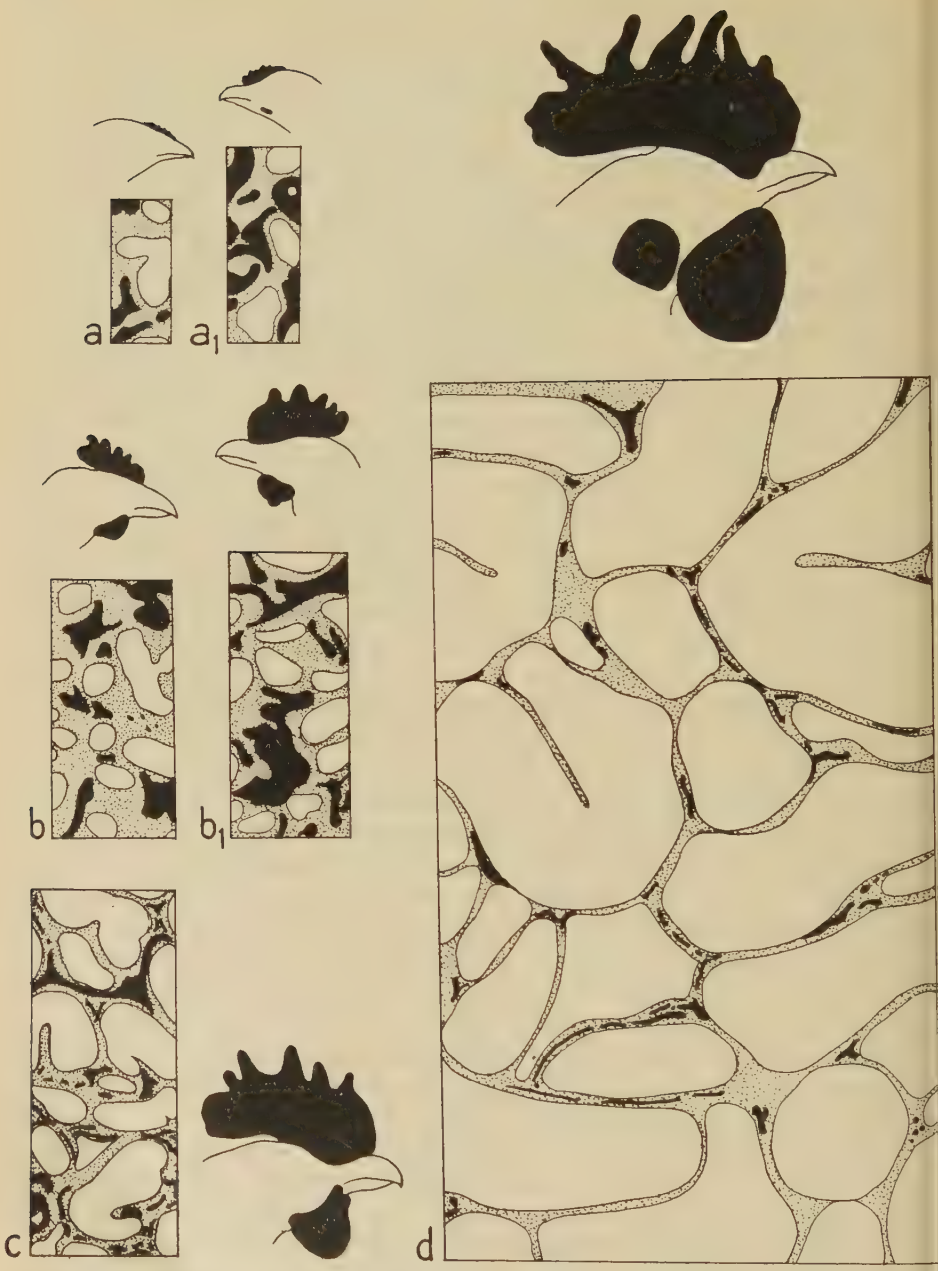
In cockerels' testes, besides intertubular connective tissue cells, nervous tissue, blood-vessels, and lymph-vessels, cells are found, the contents of which stain deeply with acid fuchsin after fixation in special fluids, e.g. Champy's fluid. They will be termed interstitial cells (in a restricted sense) and agree

with the large lipoid-storing Leydig cells, mentioned by Stieve, as well as with the glandular interstitial cells described by Benoit.

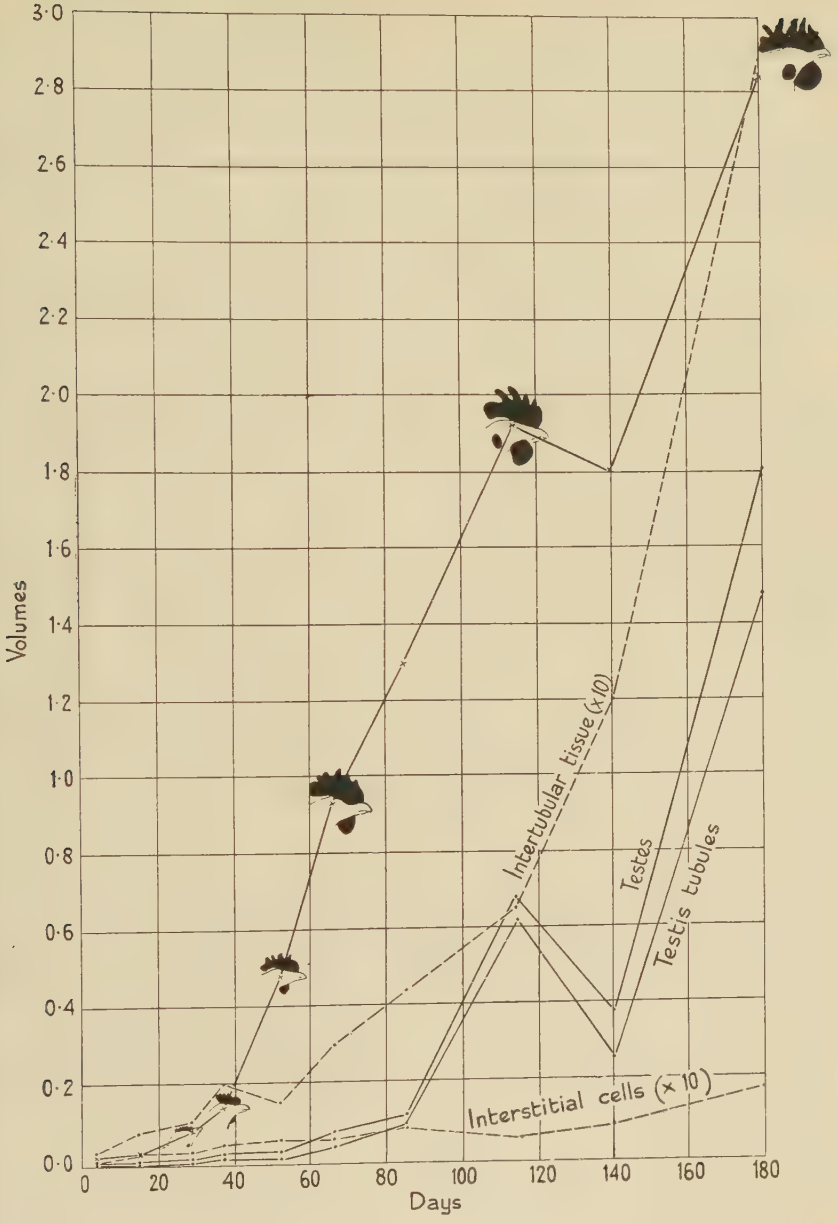
In Text-fig. 1 the sizes of the testes, of their components, and of the head-appendages of cockerels of different ages, some of them after gestyl-administration, are delineated. The average size of the testes is expressed by rectangles of which the length of the short and long side are proportional, respectively, to the average length of the short and long axes of both ellipsoid testes. In every rectangle the following testis-components are represented at the same magnification: testis-tubules (white), interstitial cells (black), and rest of the intertubular tissue (dotted). In normally developing cockerels, respectively 14, 38, 66, and 200 days old (Text-fig. 1, *a*, *b*, *c*, and *d*), the intertubular tissue is originally larger than the testis-tubules (Text-fig. 1, *a* and *b*). Later on, the latter are relatively more developed than the intertubular tissue (Text-fig. 1, *c* and *d*). The same applies to the size of all the interstitial cells taken together, compared with that of the testis-tubules. After gestyl-administration the testes of very young cockerels increase in size (cf. Text-fig. 1, *a* and a_1) contrary to those of older ones (cf. Text-fig. 1, *b* and b_1). In both cases a marked increase of the total quantity of interstitial cells is evident, an increase which runs parallel to the enlargement of the head appendages. This fact, already known in mammals, supports the hypothesis that the male sex hormone is formed in the interstitial cells (Bourg, 1930; Sluiter, 1945).

The two-dimensional drawings of Text-fig. 1 are not adequate for a comparison of the volumes of the three-dimensional testes and their components. This comparison, however, is made in Text-fig. 2. In this graph the age of the cockerels is plotted against the relative volumes of the testes and their components. These volumes have been calculated by cutting the testis-components out of the rectangular drawings, mentioned above, weighing them, and multiplying the numbers obtained by a factor, which is proportional to the average lengths of the short axes of both testes. From this graph it follows that the development of the head appendages runs about parallel with the testis-enlargement, which for the greater part must be ascribed to a growth of the testis-tubules. The distance between the curves for the growth of the testis and of the testis-tubules may serve as an indicator for the growth of the total intertubular tissue; relatively, i.e. with regard to the whole testis, this tissue decreases in size (cf. also Text-fig. 1), but absolutely it increases considerably. This enlargement is distinctly shown by the upper broken line of Text-fig. 2, which relates to the volume of the intertubular tissue, multiplied 10 times. The fact that the intertubular tissue increases in volume absolutely and that this increase runs parallel to the development of the head appendages, had already been mentioned by Benoit (1922). This fact was overlooked and sometimes even denied by investigators, who did not think it necessary to measure the quantity of intertubular tissue by a reliable method and only estimated it.

It is customary to attach much value to the volume of the total intertubular tissue in relation to the endocrine function of the testis. Benoit (1929), however, points out that only the quantity of interstitial cells is of primary



TEXT-FIG. 1. Sections of testes of normal cockerels, respectively 14, 38, 66, and 200 days old (*a–d*), and of gestyl-treated cockerels, 14 and 38 days old (a_1, b_1). The sides of each rectangle are proportional to the mean sizes of the long and short axes of both testes. Champy-fixation, Altmann's acid fuchsin-staining. × 75. Heads reduced to about $\frac{3}{8}$.



TEXT-FIG. 2. Graph showing growth of the testes, of their components, and of the head appendages in normal cockerels. Heads reduced to about $\frac{1}{12}$.

importance, because the cells which produce the male sex hormone belong to them. We entirely agree with his view. In Text-fig. 2 (lower broken line) it is shown that the volume of all the interstitial cells taken together increases slightly but distinctly and parallel to the development of the head appendages.

Summarizing, we have ascertained that parallel to the development of the head appendages the volume of the intertubular tissue as well as the total volume of the interstitial cells increases. This fact points to the endocrine function of these cells, but is no proof of it. To demonstrate the endocrine function of the interstitial cells, it is necessary to carry out an accurate quantitative investigation of the cytological structure of these cells and the functional changes which take place in them.

4. Cytology of the Interstitial Cells

As already mentioned (p. 136) it does not suffice to pay attention only to size, form, or lipoid content to distinguish the interstitial cells from other cell-types in the intertubular tissue; mitochondria, granules, and vacuoles must also be considered. Therefore the structure and possible function of the intertubular cells can only be satisfactorily studied after specific fixation and staining of the testis. The laboratory routine technique (e.g. fixation in Bouin's fluid and staining with haematoxylin) is not sufficient.

According to Benoit (1929) the interstitial cells of male chicken embryos acquire a 'chondriome plus riche' towards the end of the incubation period; then also fuchsinophil granules and vacuoles make their appearance. The vacuoles, which gradually become so numerous that they fill up the whole body of the cell, possess a lipoid content. The interstitial cells remain in this stage during the first two or three months after hatching. At the beginning of the pre-spermatogenesis stage distinct changes take place in the interstitial cells; the lipoid vacuoles disappear and large quantities of 'chondriocontes', mitochondria, and fuchsinophil granules replace them. Then, according to Benoit, these cells possess their definite structure, which they retain during sexual life.

Benoit maintains that this points to the fact that the endocrine function of the testis is located in these cells. Actually the large number of fuchsinophil elements (mitochondria, granules) points to a certain activity of these cells and it is likely that this activity is a glandular one. The presence of lipoid vacuoles is not so clear, because they sometimes occur in such quantities that there is no room for a glandular function in the cell. Stieve (1923, 1926) has already remarked that the number of these lipoid-containing cells may increase enormously under certain circumstances in adult cocks, without an increased hormone production.

Consequently several questions arise:

1. Is it possible to establish, besides the presence of the fuchsinophil elements described by Benoit (1929), other cytological properties, which point to a glandular function of certain interstitial cells? (Cf. p. 144, under 2.)
2. Does any relation exist between the lipoid occurring in the interstitial cells of cockerel-testes and the endocrine function of the male gonads? (Cf. p. 146, Conclusion 3.)

3. Is it possible to establish a well-founded quantitative relation between the activity of interstitial cells and the amount of the male sex hormone? (Cf. p. 146, Conclusion 4.)

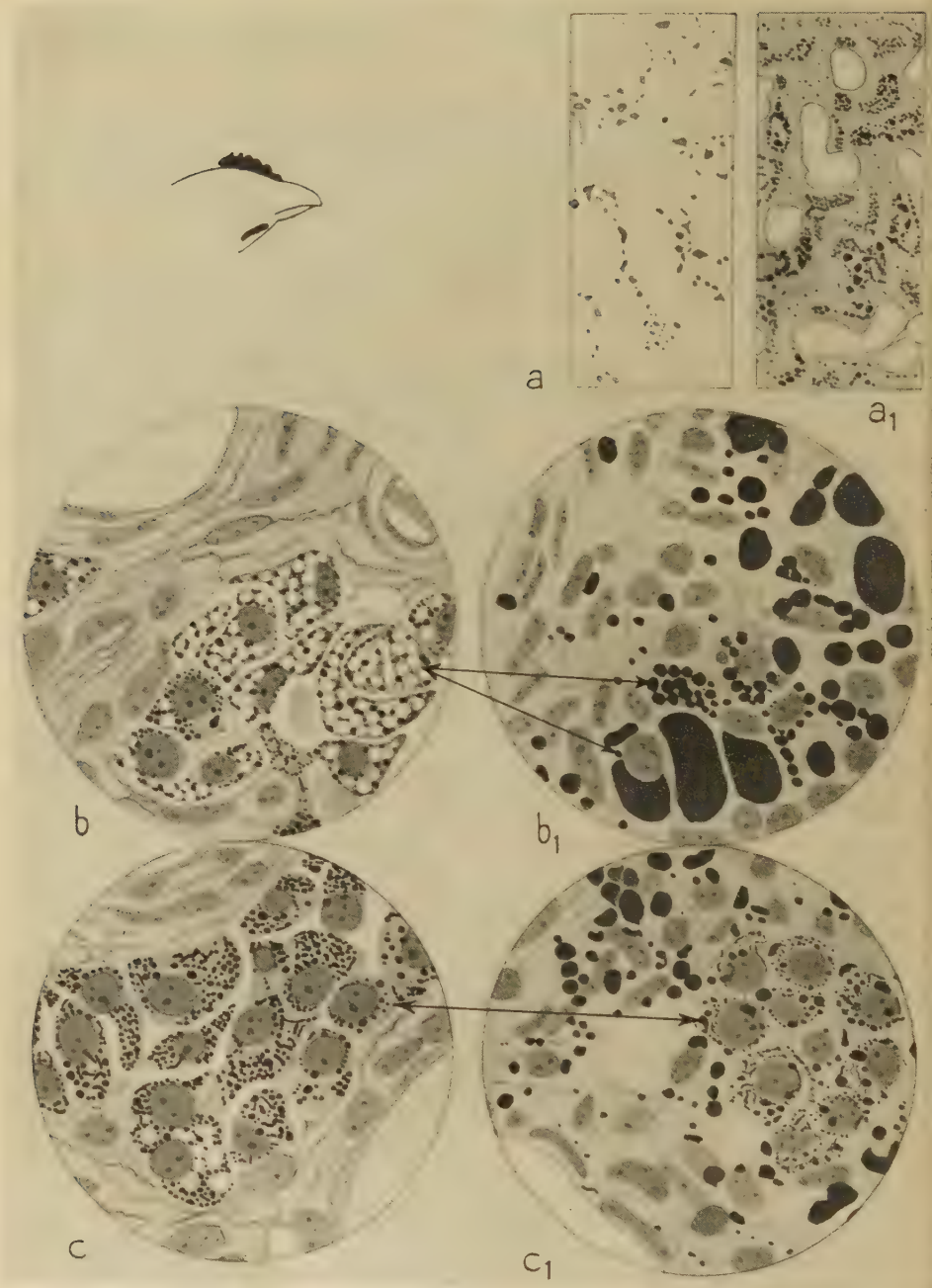
In order to answer these questions it is necessary to begin with a detailed description of the cytological structure and chemical constitution of these cells before and during the development of the head appendages and then to establish the changes occurring in these cells quantitatively, if possible by counting the cells in different functional stages.

In the first month after the cockerels are hatched, two distinctly different types of interstitial cells have been found by us in the testis (Text-fig. 3).

The *first type* relates to cells, which, after having been fixed in Champy's fluid, are almost totally filled with small vacuoles (Text-fig. 3*b*), whereas some mitochondria or other very small fuchsinophil elements are found among these vacuoles. After having been stained with Sudan III (Text-fig. $3b_1$) several cells full of red globules are visible, which are apparently identical with the vacuoles shown in Text-fig. 3*b*. It is certain that these cells are Leydig cells, which, being totally filled with fat, are storage-cells according to Stieve. We agree that in these cells lipoid is accumulated, but believe that Stieve goes too far when he concludes from its presence in these cells after Sudan-staining that this lipoid is used for the metabolism of the testis-tubules only. Therefore we have submitted these cells to Schultz's cholesterol-test. Text-fig. 3*a* shows that the blue oxycholesterol granules are only present in the intertubular spaces; on comparing Text-fig. 3*a* and Text-fig. $3a_1$—which is drawn from a Sudan-stained section—it is clear that the sudanophil substance consists at least partly of cholesterol or its derivatives. As cholesterol is the substance from which the synthetic androgens are derived, it is not impossible that the presence of a sudanophil substance in the intertubular tissue of the cockerel's testis may be connected with the growth of the testis-tubules as well as with the endocrine function of the interstitial cells. Finally we can conclude from Text-fig $3b_1$ that the sudanophil substance is found partly outside the interstitial cells, i.e. in the connective tissue cells surrounding them.

The *second cell-type* has quite another appearance (Text-fig. 3*c*). Vacuoles are lacking in these cells, which contain many, mostly granular, mitochondria. After Sudan-staining no (or very few) lipoid globules are visible (Text-fig. $3c_1$); the mitochondria are more or less sudanophil and some lipoid globules occur in the surrounding connective tissue. According to Benoit these cells are glandular cells with a marked endocrine activity.

In cockerels, *1–2 months of age*, a *third type of interstitial cell* appears (Pl. 1, *C* and *c*), characterized by the presence of one vacuole, often so large that it occupies almost the whole cell-body (Pl. 1, *C*). Mitochondria are present in rather large numbers. After Sudan-staining (Pl. 1, c_1) these vacuoles appear to possess no sudanophil contents. These cells,



TEXT-FIG. 3. Intertubular testis tissue of cockerels in the first month after hatching. *a* and a_1, respectively after Schultz's cholesterol-test and Sudan III-staining (× 100); *b* and *c*, after Champy-fixation and Altmann-staining (× 1000); b_1 and c_1, after Sudan III-staining (× 1000). Arrows refer to comparable interstitial cells. Head reduced to about $\frac{1}{2}$.

which are also strongly glandular in appearance, have apparently been overlooked by Benoit.

Using Schultz's cholesterol-test we found that the intertubular sudanophil substance in these older cockerels also mainly consists of cholesterol-derivatives (cf. Pl. 1, *a* and a_1). In several places in such a preparation

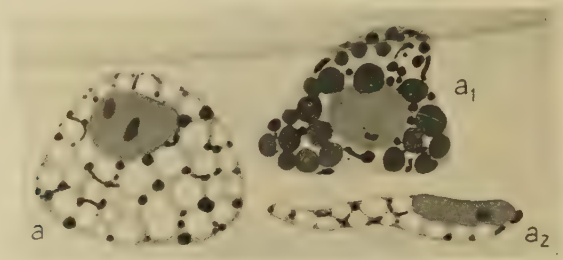
Text-fig. 4. Intertubular testis tissue of a cockerel, 200 days old. *a*, after Champy-fixation and Altmann-staining; a_1, after Sudan III-staining. × 1,000. Head reduced to about ½.

it was even possible to identify cell-nuclei and to state that the cholesterol-derivatives were lying in the cytoplasm around the nucleus (Pl. 1, a_2). We could not establish to which of the types of interstitial cells, described above, these cells belonged.

In testes of cockerels, *2–5 months of age*, all three cell-types are present. With increasing age of the cockerels small vacuoles appear in the cells of the third type in addition to the characteristic large vacuole (Text-fig. 4*a*); they possess sudanophil contents (Text-fig. $4a_1$). Moreover, the interstitial cells

of the testes of these older cockerels are smaller and more oblong than those of younger birds. This is easy to understand, as the intertubular spaces become narrower (Text-figs. 1 and 2), whereas the number of interstitial cells increases considerably.

According to Tonutti (1943) and others, the mammalian interstitial cell contains many vitamin C granules. In the testes of our cockerels the vitamin C reaction was very weak and if present vitamin C granules could be found evenly scattered over all kinds of testis tissue.



TEXT-FIG. 5. Lipoid cells from the intertubular testis tissue. Champy-fixation and Altmann-staining. ×2,250.

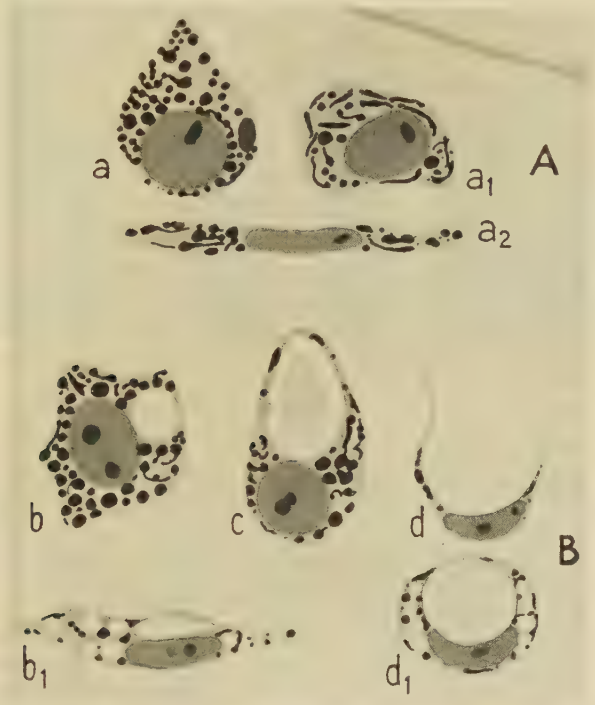
Summarizing, we can divide the interstitial cells into two essentially different types:

1. *Lipoid cells* (Text-fig. 5), characterized by sudanophil globules, which after Champy-fixation appear as empty vacuoles (Text-figs. 5*a* and 5a_2). In fresh Champy-preparations these vacuoles possess a black, i.e. an osmiophil content (Text-fig. 5a_1), which, however, dissolves after some hours in Canada-balsam. It must be emphasized that by 'lipoids' are understood several chemically diverse fatty substances and also substances which are decidedly not fats; cholesterol and its derivatives also stain with Sudan III.

2. *Secretory cells*, characterized by the presence of numerous, mostly granular (Text-figs. 6*a*, 6*b*, 6*c*) and sometimes filamentous (Text-fig. 6a_1 and 6a_2) mitochondria and the absence of lipoid vacuoles. These secretory cells are present in two cell-types: *secretory cells A* without (Text-figs. 6*a*, 6a_1, 6a_2) and *secretory cells B* with one vacuole (Text-figs. 6*b*, 6*c*, 6*d*). The contents of these vacuoles are not sudanophil nor osmiophil. After Champy-fixation the vacuole is mostly empty, but sometimes it possesses a light-refracting crystalline body (Text-fig. 6*c*). Whether this corpuscle is identical with the globuline-crystalloid of Reinke, which has been described for human and other mammalian interstitial cells, could not be ascertained.

As to the mutual relation of these cell-types, it is obvious that secretory cell-type B develops from type A, in which a vacuole, gradually increasing in size, appears (Text-figs. 6*b*, 6*c*, 6*d*). According to Benoit (1927*b*) type A is derived from lipoid cells which lose their lipoid contents and in which more and more mitochondria develop. Though we have also observed cells which

possess a rather large quantity of mitochondria in addition to many lipoid vacuoles, we have never seen a complete series of transitional stages between lipoid cells and secretory cells A. Therefore we do not think that Benoit's hypothesis is proved.



Text-fig. 6. Secretory cells. *a*, a_1, and a_2, secretory cells A; *b*, *c*, *d*, b_1, and d_1, secretory cells B. Champy-fixation and Altmann-staining. ×2,250.

5. The Number of the Interstitial Cells in Relation to the Size of the Head Appendages

From the above it follows that there are interstitial cells which may be considered glandular cells. However, this does not imply that they secrete the male sex hormone. Moreover, it is necessary to ask whether the lipoid cells play a part in producing this hormone.

In order to answer these questions we have tried to find a relation between the numbers in which the three types of interstitial cells occur, and the size of the appendages, by counting these cells in the testes of normal and gestyl-treated cockerels of different ages.

In the diagrams of Text-fig. 7 the results of these counts are shown. The numbers of the three types of interstitial cells are represented by the areas of the columns; the lightly dotted areas relate to the lipoid cells; the heavily dotted areas to the secretory cells A, and the black areas to the secretory

cells B. The ordinates represent the percentages in which these cells occur. The relative surface areas of the head appendages (comb, wattles, and ear-lobes together) are shown by the numbers given above drawings to scale of each cockerel's head.

In the upper row (Text-fig. 7*a*) the columns relate to 23 normally growing cockerels; in the lower row (Text-fig. 7*b*) pairs of columns are shown, of which the left relates to a normal control bird, whereas the right one has reference to a cockerel which has been treated with gestyl.

The data on which the columns of Text-fig. 7 are based were determined in the following way. In rectangles, similar to those described on p. 137 (cf. also Text-fig. 1) but having a surface four times larger, the number of each of the three interstitial cell-types was counted; then the total number of these cells in one testis was approximated by multiplying the number counted with a factor, which is proportional to the average length of the short axis of both testes. In this way numbers of interstitial cells were obtained, which relate to the real total number, and which can be compared with each other.

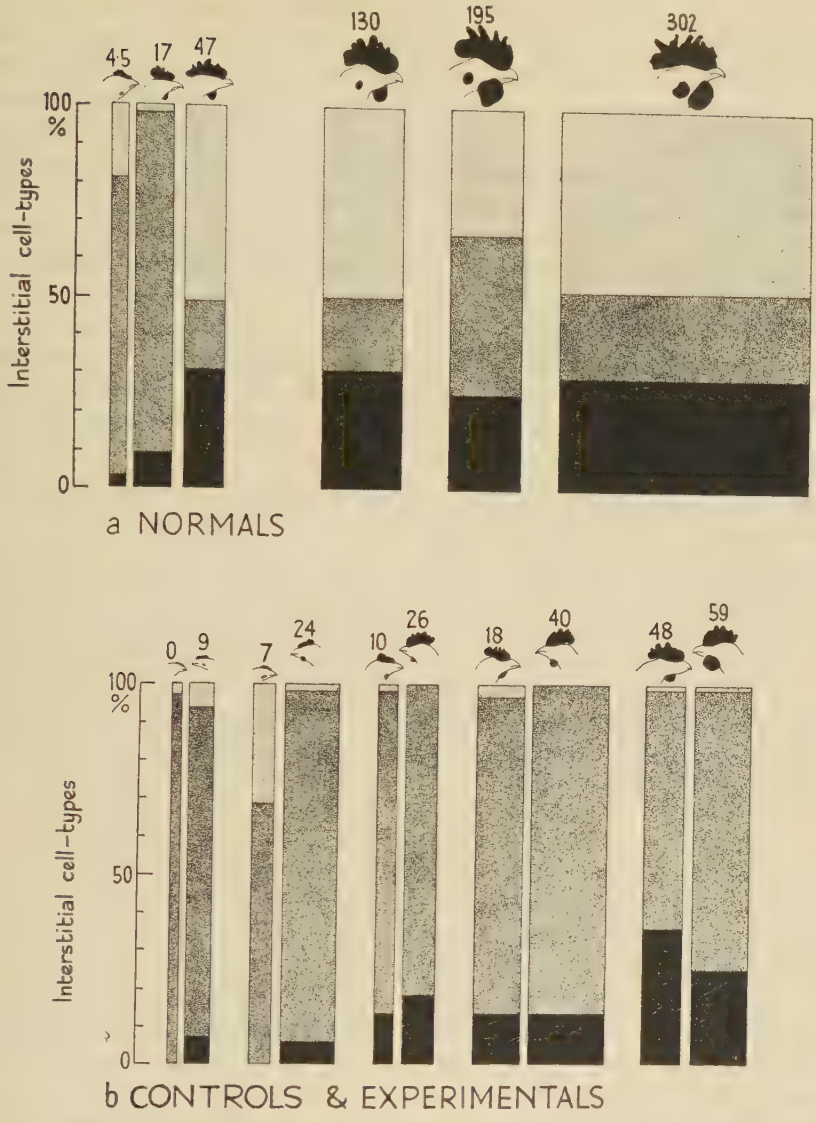
From Text-fig. 7 it follows that:

1. The total number of the three interstitial cell-types increases enormously (cf. the total areas of the columns) as the head appendages develop.
2. In normal birds about 50 per cent. of this increase in size is due to the increase in number of the lipoid cells (cf. Text-fig. 7*a*, lightly dotted areas).
3. In gestyl-treated birds this increase is only due to the secretory cells, whereas the head appendages have enlarged considerably as a result of the treatment (cf. Text-fig. 7*b*, heavily dotted and black areas).
4. In normal as well as in gestyl-treated birds, the secretory cells B (cf. Text-fig. 7, black areas) become more numerous as the head appendages increase in size. This was especially perceptible in young gestyl-treated cockerels (cf. the 2 first pairs of columns in Text-fig. 7*b*).

Conclusion 1. This fact is in favour of Benoit's opinion that the interstitial cells produce the male hormone, but it does not show which of the two cell-types, the lipoid cell or the secretory cell, is responsible for the hormone-production.

Conclusion 2. The increase in number of the lipoid cells might possibly point to the fact that the hormone is produced by the lipoid cells, but the same might be stated for the secretory cells.

Conclusion 3. From this observation it is obvious that the lipoid cells are certainly not directly necessary for the production of the hormone. The observation of Stieve (1923, 1926) who found that in fattened cocks and ganders the number of well-developed cells of Leydig (i.e. our lipoid cells) increases enormously whereas the head appendages do not increase in size, is also in favour of this opinion. Furthermore, one can deduce from Text-fig. 7*a* that the number of lipoid cells increases as the testis tubules develop. This suggests that the testis-tubules do not absorb substances that have been stored



TEXT-FIG. 7. Diagrams showing relative numbers of lipoid cells (lightly dotted), secretory cells A (heavily dotted), and secretory cells B (black) in normal cockerels of 2–200 days (*a*) and in gestyl-treated cockerels. In *b*, the right column of each pair of columns refers to a gestyl-treated cockerel of 14–52 days; the left column refers to a normal control bird. The total numbers of interstitial cells and of each cell-type are shown respectively by the surface area of each column and of its components. The numbers above each head show the relative surface area of the head appendages.

in these lipoid cells. Therefore the quantity of lipoid cells in the testis interstitium seems to depend only on food conditions of the animal, i.e. on the quantity of lipoid that the body can spare for storage at a certain moment.

Perhaps the lipoid cells are indirectly connected with the formation of the hormone, as they might act as storage-cells of special elementary substances. That they contain cholesterol-derivatives (cf. p. 141) points in this direction.

Conclusion 4. The fact that the secretory cells B, representing the most advanced functional stage of secretory cells, increase in number as the head appendages develop, in normal as well as in gestyl-treated animals, is especially important in proving that the secretory cells produce the male sex hormone. The formation of one large vacuole in each cell is in agreement with this. Moreover, the number of secretory cells B is a standard for the quantity of hormone produced.

According to Benoit (1927) the presence of only 0·3 gm. of testis-tissue is sufficient for the complete development of the cock's comb. However, the testes of the cockerels represented by the last column of Text-fig. 7*a* weigh much more. Therefore the large quantity of secretory cells in the testes of these birds points to the following possibilities:

1. In older cockerels there is a large overproduction of the male hormone, this being, however, improbable;
2. Each of the secretory cells B secretes much less hormone in older than in younger birds. This supposition agrees with the fact (cf. p. 143) that, as the cockerels grow older, i.e. from the age of 2 months onwards, lipoid granules originate in these cells. In our oldest cockerels this was the case in most of the secretory cells B. Therefore these cells have the appearance of storage cells (cf. Text-fig. 4 and $6b_1$, $6d_1$) and only one large conspicuous vacuole reminds one of their original glandular function.

6. Discussion

Finally we will try to give a better insight into the function of the interstitial tissue in cockerels than has hitherto been possible.

It cannot be denied that the storage of lipoids is one of the functions of the interstitial tissue.

The investigations of Benoit have, above all, shown that the endocrine function of the cock's testis must be localized in the interstitial tissue and that glandular cells are found in it. Moreover, the occurrence of the so-called secretory cells B with their large vacuole is in favour of this opinion.

Benoit has also demonstrated a parallelism between the glandular activity of the interstitial tissue and the quantity of male hormone produced. He drew this conclusion from the fact that the total quantity of the interstitial cells increases proportionally to the enlargement of the head appendages, but he does not distinguish the glandular cells with many mitochondria and little lipoid from the lipoid cells, both being considered as secretory cells.

On the contrary, our counts of the interstitial cell-types have shown that Benoit's view, though perhaps not wholly at variance with reality, is much too schematic: there is no reason to call cells, which are packed full of lipoid globules, glandular cells; true connective tissue cells may show the same

phenomenon. In case of the interstitial cells it is therefore likely that these lipoids have only been stored and have not been produced by these cells.

Even if this should be the case, it is not clear what relation exists between the extensive lipoid storage and the formation of the sex hormone. For, in our gestyl-treated cockerels, which had a marked hormone production, lipoid vacuoles in the interstitial cells were practically lacking.

Stieve's fattening-experiments are also in favour of our opinion that the number of the typical lipoid cells relates to the quantity of lipoids which the organism can spare for storage. Therefore we are of opinion that only the secretory cells A and B are responsible for the endocrine function of the testis, an opinion especially founded on the observation that after gestyl-injection the number of these cells alone runs parallel to the enlargement of the head appendages.

Summarizing, we conclude that in embryos and newly hatched male chickens the interstitial testis-tissue has only a trophic function, which is amongst other things evident from the lipoids stored in special lipoid cells, in connective tissue cells and also intercellularly. Then, as the development of the head appendages becomes visible, glandular cells appear which produce the male sex hormone. The storage of intercellular lipoid granules still occurs and lipoid cells are still present at this age. We can neither confirm nor deny Benoit's opinion (1929) that the glandular cells develop from lipoid cells.

During further development, i.e. in cockerels of 1–2 months old, the glandular cells increase in number and become more active, which is shown, e.g., by the formation of a large vacuole in the secretory cells B. In cockerels older than 2 months many of these cells undergo a regression and pass over into lipoid cells.

Finally the situation in the adult cock is reached: only a small percentage of the original secretory cells still function as such, and the others have become storage cells. In old cockerels and adult cocks the accumulation of lipoids is mainly intracellular.

Our opinion confirms that of Benoit (1927*a*), according to whom a 'secretion de luxe' of testis hormone never takes place. However, we cannot agree with Benoit when he accepts a 'parenchyme de luxe' in cockerels with a testis-weight of more than 0·3 gm. For the tissue, which this author claims to be superfluous, really consists partly of cells which have had a secretory function, but which now possess another function, i.e. the storage of lipoids.

7. Summary

1. The relative volumes of the testes and their components of 31 cockerels, 2–200 days old, were calculated and compared with the size of their increasing head appendages (Text-figs. 1*a–d*, 2); in addition, the effect of gestyl-administration on testes of cockerels of this age was investigated.

2. Several types of interstitial testis-cells could be distinguished morphologically and physiologically (Text-figs. 3–6 and Pl. 1); these cell-types were studied with different techniques and counted separately.

3. The main types of the interstitial cells are:

(*a*) Lipoid cells, totally packed with lipoid globules. These cells, which are considered by many authors as fully developed Leydig cells, are not directly connected with the production of the male sex hormone; perhaps they have a secondary function in this respect, as cholesterol-derivatives are stored in these cells (Pl. 1, Text-fig. 3*a*).

(*b*) Secretory cells, characterized by the absence of lipoid vacuoles and the presence of numerous granular and filamentous mitochondria. These secretory cells, which produce the male sex hormone, can be divided into secretory cells A (Text-fig. 6*a*) without, and secretory cells B with, one large vacuole (Text-figs. 6*b*, 6*c*, 6*d*).

4. A considerable and partly intercellular storage of lipoids may take place at any age in the intertubular connective tissue (Text-figs. 3–4 and Pl. 1).

5. The number of the lipoid cells depends on the nutritive conditions of the animal and the development of its testes (Text-fig. 7).

6. In older cockerels most of the glandular cells lose their secretory function and pass over into lipoid storing cells.

7. Therefore we agree with Benoit, when he denies the occurrence of a 'secretion de luxe', but we cannot accept the presence of a 'parenchyme de luxe' in the testes of older cockerels.

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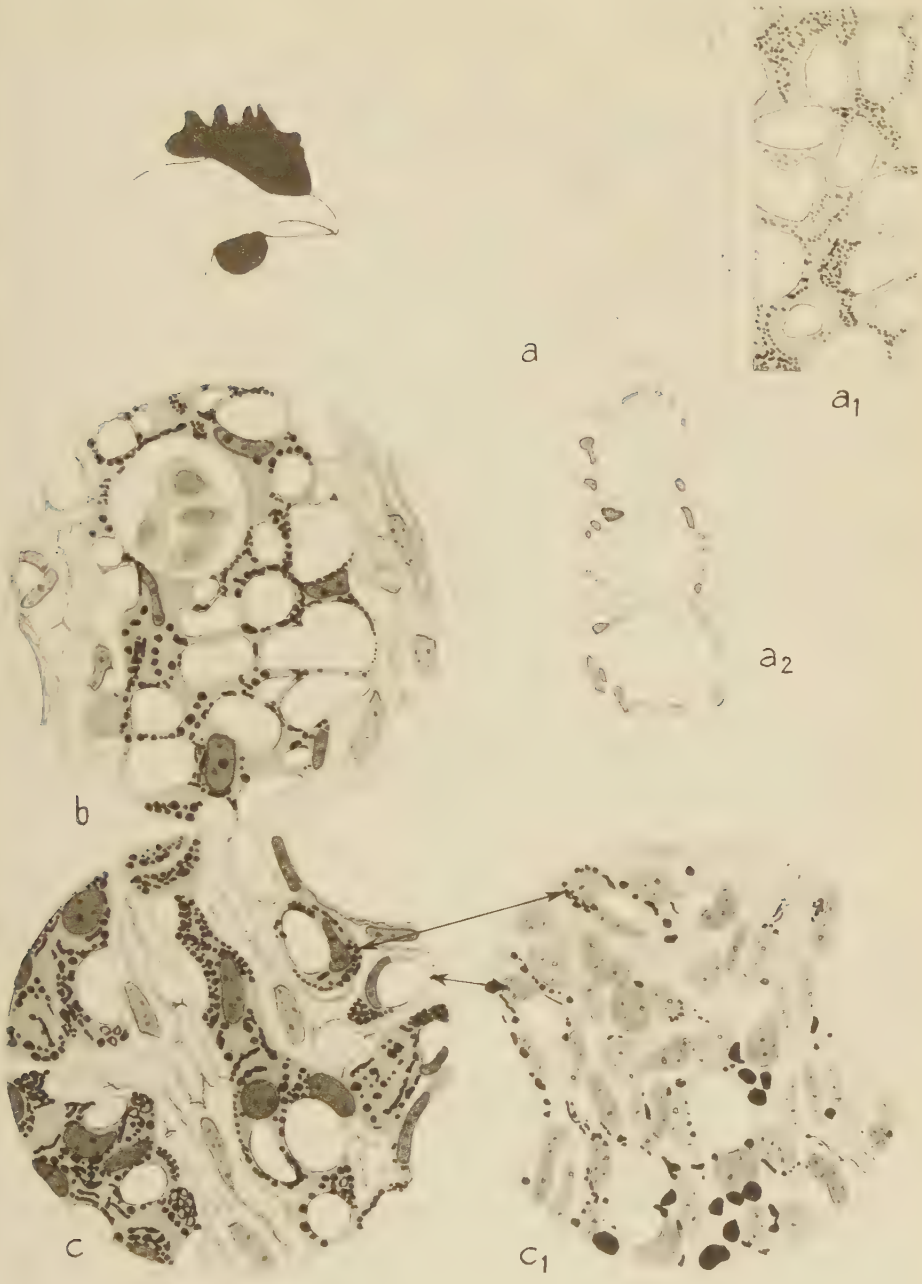
DESCRIPTION OF PLATE

Plate 1. Intertubular testis tissue of cockerels, about 50 days old. *a* and a_2, after Schultz's cholesterol-test; a_1 and c_1, after Sudan III-staining; *b* and *c*, after Champy-fixation and Altmann-staining. *a* and $a_1 \times 100$; $a_2 \times 1{,}500$; *b*, *c*, and $c_1 \times 1{,}000$. Head reduced to about $\frac{1}{2}$.



J. W. Sluiter and G. J. van Oordt

PLATE I

Demonstration of Lipine in the Golgi Apparatus in Gut Cells of *Glossiphonia*

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With two Text-figures

Introduction

THE Golgi apparatus has long been thought to consist in part at least of lipoids. Baker (1944) has summarized and discussed the evidence. The fact that it can be coloured by lipoid colorants (sudan black and sudan III) is a proof that a lipoid or lipoids are present in it. Baker has given very strong evidence, not amounting to histochemical proof, that lecithin or cephalin (or both) are present. The opinions of other workers on the nature of the lipoid contained in the Golgi apparatus are not based on reliable tests.

The purpose of this paper is to present histochemical proof that the Golgi apparatus in the epithelial cells of the alimentary canal of the leech, *Glossiphonia complanata*, contains lipine.

In this paper, the word 'lipoid' is used to include fats and all other substances that occur in plants and animals and resemble fats in solubility. The word 'lipine' refers to lipoids that yield fatty acids, phosphoric acid *or* galactose, and a basic nitrogen compound.

Material

This study was made on the gut epithelium of the Rhynchobdellid leech *Glossiphonia complanata* (L.). This animal was originally chosen for the suitability of its fat-cells for work on lipines, and because it is common and occurs throughout the year in the adult stage. The Golgi apparatus is visible in the gut after formaldehyde-calcium fixation in unstained preparations mounted in Farrants's medium, and is very easy to demonstrate by a variety of methods. It appears to have the same structure in both stomach and intestine, but although it is easily shown in both by sudan black, the standard silver and osmium techniques give good preparations far more often with the intestine than with the stomach. On the other hand, the acid haematein test rarely gives good results with the intestine, partly because of interference by a diffuse lipine in the cytoplasm.

Methods

For definitive demonstration of the Golgi apparatus, the following standard techniques were used:

Silver: Da Fano
Ramon y Cajal
Aoyama

Osmium: Mann-Kopsch-Weigl (Ludford)

These were supplemented by Kull's method, after Helly fixation, for mitochondria.

For investigating composition, Baker's acid haematein test for lipines (Baker, 1946) was used, and the following method for sudan black:

(1) Fix, postchrome, wash, embed in gelatine, and cut frozen sections exactly as for the acid haematein test.
(2) Leave for a few minutes in 50 and 70 per cent. alcohol.
(3) Transfer to a saturated solution of sudan black in 70 per cent. alcohol for 7 minutes or longer (the exact time does not matter; see Lison, 1936, Baker, 1944).
(4) Pass through three lots of 50 per cent. alcohol, 30 seconds in each.
(5) Rinse in distilled water and mount in Farrants's medium, or counterstain first if desired. One counterstained preparation is useful if the tissue is not familiar.

This method is a modification of Baker's. It was used in preference to Lison's because Dr. Baker informed me that the use of potassium dichromate helps to make the Golgi apparatus colourable with sudan black in certain cases in which it is not shown by Lison's technique.

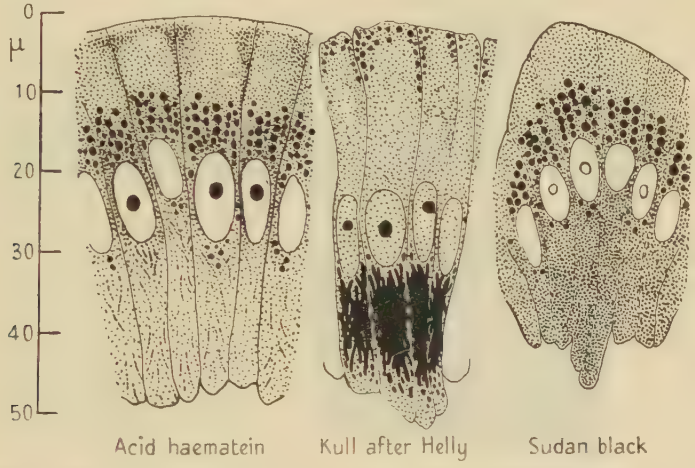
It was found that no difference could be seen between preparations made in this way and those fixed in formal-calcium with no postchroming, so the same block was used for acid haematein and sudan black.

The method for nile blue given by Lison (1936) was used, except that again, as there was no difference between postchromed and not-postchromed sections, postchromed ones were used. Also, a variety of tissues (sections of *Glossiphonia complanata*, *Lumbricus castaneus*, *Dendrocoelum arboreum*, and liver, lung, and kidney of mouse) were stained in a saturated aqueous solution boiled with $\frac{1}{2}$ per cent. sulphuric acid (as advised by J. L. Smith, 1908, and recommended by Lison, 1935*b* and 1936), and compared with others stained in the untreated solution. Again, no difference could be seen, so the untreated solution was used, as it appears to keep indefinitely. In addition, the following method was used.

(1) Prepare a section as for acid haematein.
(2) Stain in saturated aqueous solution of nile blue, 10 minutes.
(3) Leave in 1 per cent. acetic acid for about 18 hours, until the Golgi apparatus is pale pink and the cytoplasm colourless.
(4) Wash in distilled water, and mount in Farrants's medium.

Lison (1935, *a* and *b*) concludes, after a very detailed study of nile blue, that the red coloration is due to nile red, an oxazone occurring as an impurity in nile blue, and that this behaves like any other lipoid-colouring agent,

e.g. sudan black. It cannot distinguish between the various classes of lipoid. (This conclusion, as Kay and Whitehead (1937) point out, supersedes that given in his book (1936).) He states also that the blue dye is simply a basic dye, and no histochemical conclusion whatever can be drawn from its uses, and (1935*a*) that it has weak powers of metachromatic staining with ordinary chromotropic elements, the metachromatic colour being *violet bleuâtre*. The method with prolonged differentiation given above does enable one to distinguish between the metachromatic and allochromatic colorations in some cases. It is believed, from some unpublished work, that Lison's conclusions



TEXT-FIG. 1. Parts of transverse sections of the stomach of *Glossiphonia*.

are a little over-pessimistic, and that under certain well-defined conditions distinctions can be drawn between certain classes of lipoid; this will be the subject of a separate paper. In the meanwhile, it must be emphasized that no more specificity is claimed for this method than that a red coloration with an aqueous solution of nile blue does show a lipoid. Its value here is purely morphological, as an adjunct to the sudan-black method.

These methods are further discussed below.

RESULTS

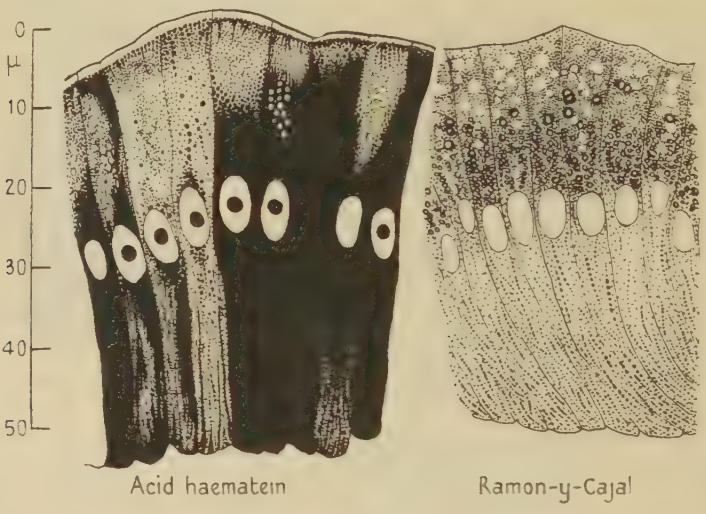
(I) *Description of the Golgi apparatus in the alimentary canal of* Glossiphonia

The stomach and intestine of *Glossiphonia* are both very distensible, and the shape of the epithelial cells varies greatly, so that the differences figured by Brumpt (1900) are not always as clear as one might wish. The surest distinction is the position of the lateral diverticula relative to the testes. Those of the stomach lie between or outside the testes, those of the intestine above them.

When the alimentary canal is not distended, both epithelia may be described as columnar (Text-figs. 1 and 2). The nucleus, with a conspicuous

plasmosome, lies in the middle region of the cell. The mitochondria are massed at the base of the cell (most proximal part) and may occupy almost the whole of that region up to the nucleus, but a few are scattered in the distal area, chiefly away from the Golgi region, and close to the sides of the cell. The main mass of the Golgi apparatus, in both epithelia, lies immediately distal to the nucleus (that is, on the side towards the lumen of the gut), but parts may extend down its sides, or even surround it.

The constituents of the Golgi apparatus, as shown in undistended epithelia by the standard methods, appear as numerous uncoloured globules, nearly



TEXT-FIG. 2. Parts of transverse sections of the intestine of *Glossiphonia*.

every one of them surrounded, at least in part and usually completely, by a shell of strongly osmiophil and argentophil substance. Almost all are arranged in lines parallel with the long sides of the cell. If the globules in one cell are more or less uniform in size, then that size is at the lower end of the range of variation seen. If they are not uniform, then usually a wide range of size is seen, the largest having no shell and being in the most distal part of the cell. The rest of the cytoplasm in osmium preparations is a general grey, usually rather darker in the region of the mitochondrial mass. In toned silver preparations this area is definitely darker than the rest of the cytoplasm. There is no definite evidence for a diffuse osmiophil or argentophil substance specially concentrated in the Golgi region.

The standard techniques usually fail to show anything in the stomach epithelium, but are quite reliable for the intestine. A good Da Fano stomach preparation was obtained, but the Mann-Kopsch technique usually failed completely; and in the only good preparation obtained, the stomach was in the distended state, and the cells so reduced in height that interpretation was very difficult.

(II) *Composition of the parts of the Golgi apparatus*

In both epithelia, after coloration with sudan black, the Golgi apparatus is seen as a crowd of black, usually nearly spherical bodies, arranged in rows as before; occasionally some are apparently coalescing. There is no special indication of a diffuse lipoid between them. A colourless internum is not to be seen.

After acid haematein, it is seen that in the stomach the Golgi apparatus is stained blue quite deeply, the mitochondrial mass much less so. The cytoplasm is greyish from the Golgi area to the free edge, colourless or faintly yellowish elsewhere. The nucleus is almost colourless, except for the plasmosome, which varies from brown to black. The pyridine extraction test gives a completely negative result (except for the nucleus and plasmosome) in both stomach and intestine. As before, the apparatus takes the form of spherules or nearly spherical bodies arranged in rows, more or less, and again, it is difficult to see in any a definite internum. In the intestine, an altogether different appearance is seen. Staining with acid haematein is by far the best method of distinguishing between these two parts of the alimentary canal if there is any doubt. Almost every intestinal cell is full of the blue stain, sometimes so much so that it seems black rather than blue. The plasmosome is again stained, more usually black than brown, and the rest of the nucleus is the only transparent part of the cell. It is usually pale-yellow or yellowish-brown. Nearly always, the whole of the intestine and its diverticula that can be seen in the section is stained like this (and, on a few occasions, the most posterior part of the posterior pair of stomachic diverticula may appear the same), but sometimes a cell here and there, or a small group of cells, is seen to be comparatively clear. In that case the mitochondrial mass is still very darkly stained, and there is a blue patch just inside the free border; but the Golgi region is almost unstained. Often spherules can be seen, but by refraction; a few grains are usually stained, but are very small, and extremely like the small mitochondria in the most distal region. Sometimes, when the whole cell is stained blue, small clear bodies can be seen clustered in the Golgi region. They resemble, quite closely, those seen in that region in unstained sections.

Staining with nile blue by Lison's method shows the Golgi apparatus in both stomach and intestine as spherules (or nearly spherical bodies), darker blue than the surrounding cytoplasm and slightly redder in tone. (As globules of fat in the cell, if present, are coloured red, so that confusion is very unlikely, this is a good, simple method for demonstrating the Golgi apparatus.) Their disposition in the cell is as already described. After prolonged differentiation, quite another picture is seen, but unfortunately a rather faint one. The cytoplasm is now colourless or slightly yellow, the nucleus a very pale blue. The spherules of the Golgi apparatus are seen as colourless or faintly pink globules with definitely pink shells. The internum can now be distinguished clearly from the externum, and it appears to be the latter which is coloured. Under the low powers only, a general diffuse

pinkness is seen in the Golgi region; this is probably due to the colour in the shells of spherules that are out of focus.

Discussion

Lison (1936), speaking of the use of the lipoid colorants, says: 'La spécificité de ces méthodes est parfaite; seuls les lipides donnent des réactions positives.' Nevertheless, if a piece of paper be coloured with sudan black by either Baker's or Lison's methods, it will be seen to retain a faint coloration. And the same depth of colouring will be produced on paper that has first been soaked in pyridine for 24 hours at 60° C.—a procedure that, after suitable fixation, in Baker's pyridine extraction control test results in removing all lipoid except the faintest trace of lipine in the fat cells from a piece of *Glossiphonia*, as may easily be seen by taking a section prepared by the pyridine extraction test method and colouring it with sudan black, instead of staining with acid haematein in the regular way. With such a section, sudan IV, used in the same way as sudan black, gives a quite definite general pink tinge, as it does with paper. It seems reasonable to conclude that if a tissue is not coloured by the very powerful sudan black, a coloration by sudan IV does not show a lipoid. The general pale-pink tinge given by sudan IV has no significance. It is possible, I suppose, that such a powerful colorant as sudan black may actually be colouring an absorbed lipoid, not removable by pyridine, on the paper. However, for very faint colouring by sudan black there remains a slight doubt, unless it disappears after pyridine extraction. As the gut cells of *Glossiphonia* are completely uncolourable by sudan black after pyridine extraction, the point does not arise in this case.

The coloration of paper by sudan black was first noticed by Dr. J. F. A. McManus, who pointed it out to me in conversation.

Nile blue preparations made by the method quoted by Lison show the Golgi apparatus quite clearly, in a darker and slightly redder blue than the surrounding cytoplasm. Tarao (1939) noticed that it was 'blue with red tone' in the liver cell of the mouse. The result with prolonged differentiation makes it clear that this is principally, if not entirely, a case of allochromasy, not metachromasy; but this method is chiefly valuable for showing that the lipoid substance is greatly concentrated in, or confined to, the shell or externum. This makes it very probable that the only reason why the internum was not visible in the sudan black preparations was that the shell was so strongly coloured as to be opaque. That at least the principal lipoid in the Golgi apparatus of the stomach cells is lipine is shown by the positive result with Baker's test. It is suggested, therefore, that the Golgi apparatus in the stomach is composed of rows of (comparatively) fat-free globules with lipoid shells containing lipine.

In the intestine, as exactly the same picture is obtained with sudan black and the standard techniques, the structure appears to be the same as in the stomach, but with this important difference, that where the Golgi apparatus is visible after acid haematein, it is seen to contain little or no lipine as shown by

that test. That it contains much the same amount of lipoid as the stomach Golgi is seen from the sudan black preparations; and as the largest globules, nearest the free border, are not osmiophil or argentophil, it seems likely again that the opacity of the spherules with sudan black is due to intensity of coloration, as in the stomach. It is worth noting that the amount of lipine in the rest of the cell seems to vary inversely with that in the Golgi, there being very little in the stomach cells, and usually a great deal in the intestine. Nevertheless, the bulk of mitochondrial substance appears to be much the same in both. As lipine appears to be particularly plentiful in the actively metabolizing nephridial cells (where it is confined to the very long and numerous mitochondria), and in the fat-cells, where it occurs throughout the cytoplasm, it might be suggested that the abundance of lipine in the intestinal cells is correlated with the active role of the intestine as the food-absorbing region (see Brumpt, 1900).

Acknowledgements

My grateful thanks are due to Dr. J. R. Baker, who supervised the work recorded in this paper, and gave me great assistance, and to Dr. J. F. A. McManus, with whom it was discussed in part.

Summary

(1) The standard silver and osmium techniques show a typical Golgi apparatus in the epithelial cells of the stomach and intestine of the leech, *Glossiphonia complanata*.

(2) Histochemical studies reveal the presence of lipoids in the Golgi region of these cells. In the stomach, part, at least, is lipine. (In the intestinal cells, the rest of the cytoplasm contains much lipine, but the Golgi apparatus little or none that can be shown by the method used.)

(3) The Golgi apparatus in these cells appears to consist of rows of non-lipoidal spheres, each with a lipoid coat. In the stomach, this coat contains lipine.

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Note on the Cytological Localization of Alkaline Phosphatase

BY

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IN comparing various techniques for the microchemical demonstration of alkaline phosphatase it was observed that with the method of Menten, Junge, and Green (1944) nuclear phosphatase cannot be demonstrated in epithelial cells, whereas with the Gomori–Takamatsu (1939) technique both nuclear and cytoplasmic elements (e.g. brush borders and the Golgi zone) of suitable organs show the presence of the enzyme. The substrate in the method of Menten, Junge, and Green is Ca α- or β-naphthylphosphate in the presence of α-naphthyl-diazonium hydroxide. In the Gomori technique the substrate is sodium-β-glycerophosphate. The failure of nuclear phosphatase to be demonstrated by the first method might be caused by:

I. Failure of nuclear phosphatase to act on Ca α-naphthylphosphate.

II. Inactivity of nuclear phosphatase at the low temperature (10°) at which the diazo mixture has to be kept.

III. Irreversible inactivation by one of the constituents of the diazo mixture or

IV. Inhibition by their presence during incubation.

V. Failure of the diazo method to detect the relatively low concentrations of phosphatase in the nuclei.

To ascertain which of the above postulates is correct the following experiments were performed: Paraffin sections of guinea-pig small intestine and rat kidney fixed in 80 per cent. alcohol were placed in the substrate of Menten, Junge, and Green at 10° C. for 4 hours.

This mixture tends to lose its activity, by-products being precipitated, and was therefore renewed after 2 hours. Other slides were incubated in Gomori's substrate at the same temperature. All media were at pH 8·5 and contained 0·2 per cent. $MgCl_2$.

The sections incubated in the diazo mixture showed a heavy deposit of dye in the free border of the intestinal epithelium and of the convoluted tubules of the kidney cortex, the nuclei being negative. The Gomori slides showed blackening of the nucleoli and the nuclear membrane as well as of cytoplasmic elements. Thus nuclear phosphatase does act on glycerophosphate at 10° C., and postulate II does not apply.

One of the slides was now transferred from the diazo to the Gomori substrate at the same temperature and incubated for 4 hours. The same degree of blackening of the nuclei was produced as in the sections not given a prior incubation in Menten, Junge, and Green's medium, showing that the activity of nuclear phosphatase is unimpaired by previous contact with the diazo mixture (III).

To investigate cause IV experiments were done to see whether α-naphthyldiazonium hydroxide (*a*) or Ca α-naphthylphosphate (*b*) inhibit the hydrolysis of glycerophosphate (*c*) and whether glycerophosphate inhibits the hydrolysis of Ca α-naphthylphosphate. It was found that (*a*) inhibits hydrolysis of (*c*) and (*c*) inhibits hydrolysis of (*b*) as indicated by the lack of formation of precipitates in the sections. It must be noted that cytoplasmic as well as nuclear elements failed to blacken. But this lack of precipitation does not necessarily signify, in this instance, failure to hydrolyse: it may only indicate that the products of hydrolysis are not precipitated under these conditions.

It has been shown that phosphatase in the striated border of kidney tubules is more easily destroyed by heat than nuclear phosphatase (Danielli and Catcheside, 1945). There is thus considerable evidence that nuclear and extranuclear phosphatase behave as different enzymes in the given epithelia. Moreover, Dempsey and Deane (1946) have demonstrated cytochemically that a number of phosphatases acting on different substrates coexist in the duodenal epithelium.

The above results are in contradiction to Menten, Junge, and Green's (1944) statement that the distribution of alkaline phosphatase in the kidney appears to be the same whether their method or that of Gomori be used.

However, the fact that polymorph nuclei in glomerular capillaries of guinea-pig kidney sections show deposits of azo-dye is in favour of postulate V, viz. that there might be insufficient nuclear phosphatase in the epithelial cells examined. Danielli (1946) in a critical study of histochemical methods relating to phosphatase suggests that the diazo technique might be inferior to the Gomori method in revealing sites of low phosphatase activity. To investigate this further, sections known to be very rich in nuclear phosphatase (as judged by a heavy deposit after short periods of incubation with Gomori's substrate) were treated by the diazo method. The following tissues were chosen: epiphyses of long bones of growing mice and the costo-chondral junctions of rat ribs (decalcified by my method [Lorch 1946]), calcifying cartilage of dogfish embryos, and kidneys of various fish known to contain much nuclear phosphatase in the interstitial cells. In all tissues examined some of the nuclei were positive.

Summary

It is concluded that there is no definite proof that nuclear phosphatase as such is qualitatively different from extra-nuclear phosphatase, but that the method of Menten, Junge, and Green is not capable of revealing sites of low concentration of phosphatase in which respect it is markedly inferior to the Gomori–Takamatsu technique.

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The Use of Cajuput Oil in Microscopy

BY

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DURING the world war 1914–18 our stock of xylene (xylol) in Java ran low; moreover, the small quantity that was left changed into a thick syrupy fluid which was useless as a medium for mounting water-fixed preparations in balsam. Chemists told us that the xylene had polymerized. In text-books of microscopical technique cedar-wood oil is recommended as a substitute for xylene in some cases. However, this oil was still scarcer in Java than xylene; therefore we resolved to try another ethereal oil, namely, cajuput oil. Of this oil, a product of the Malay Archipelago, unlimited quantities were available. It soon proved to be not only a good substitute for xylene, but in some cases superior to it.

Cajuput or cajaputih oil is obtained by distillation from the leaves of *Melaleuca leucadendron* L. (*Melaleuca cajuputi* Roxb.). 'Cajaputih' is apparently a misnomer for the Malay *kaju putih*, i.e. white wood. The oil is manufactured in the island of Buru (Moluccas) and, to a less extent, in the western part of the neighbouring island of Ceram. The oil on the market is green, owing to a small admixture of copper salts derived from the distillation apparatus. Oil containing no copper is pale brown. In the Far East, particularly in the Malay Archipelago, it is a very popular medicine for almost any ailment. As a rule it is applied externally for massage, but a few drops may be swallowed in cases of abdominal complaints. Being accustomed to it, the patients ask for the green oil; the brown is not popular, and so only the green oil containing copper can be had on the market. According to Lee and Mayer (1898) and to Ehrlich (1910) cajuput oil is recommended by Carnoy and Lebrun and by Nissl as an intermedium before imbedding in colophonium.

A real advantage of cajuput oil over xylene is its ability to absorb small quantities of water. If films or sections are passed from alcohol to balsam via xylene, the alcohol must be nearly free of water; otherwise water is deposited in the preparation and renders it cloudy. Now in the moist and warm climate of Java it is very difficult to keep alcohol water-free, even with burnt copper sulphate on the bottom of the container. On the other hand, slides may be transferred from alcohol, containing a small amount of water, to cajuput oil and afterwards to balsam without getting turbid.

The capacity of cajuput oil to absorb small quantities of water proved to be useful especially in mounting wet-fixed, Giemsa- (or Leishman-) stained preparations. It is usually recommended to pass such slides successively

through: (1) acetone 95 p., xylene 5 p.; (2) acetone 70 p., xylene 30 p.; (3) acetone 30 p., xylene 70 p.; and (4) pure xylene. Slides ought to remain for a very short time only in the fluids containing acetone as otherwise the preparations are decolorized. Sometimes too much stain is extracted before complete dehydration. If cajuput oil is used the slides may be passed into the oil after a very short stay in acetone (say, one second); after which they may remain many minutes in the oil for complete dehydration without becoming decolorized. The degree of decolorization of overstained films can be watched easily by mounting them in cajuput oil under a cover-glass; if insufficiently decolorized they may be passed at once for one or more seconds to acetone.

Another advantage of cajuput oil over xylene I experienced when mounting chitinous structures, mainly hypopygia of Culicids, in balsam. Not only is turbidity by deposit of water avoided, but also the oil does not render the chitin brittle. This is important, because the position and the arrangement of the parts of the hypopygium will often have to be corrected with a dissecting needle after it has been transferred to a drop of balsam. Xylene-treated chitin is very brittle; even a slight touch may damage it. This will not happen if xylene is substituted by cajuput oil. For the same reason the oil may serve as an intermedium between alcohol and paraffin in imbedding chitinous structures in the latter. Moreover, in this case it has the advantage of being more volatile than, e.g., cedar-wood oil. Therefore it is more easily eliminated from the paraffin by heating. Paraffin sections still containing a trace of cedar-wood oil will expand greatly when flattened on water.

It is quite possible that in the above-mentioned cases other ethereal oils are as good as cajuput oil. At any rate the latter is one of the least, if not the least, expensive. Before the last war the price of 1 litre (1·76 pints) in Amsterdam was fl. 1·70 (i.e. about 3*s.* 6*d.*).

Summary

Cajuput oil has some advantages over xylene for mounting preparations in balsam. Cajuput oil can hold some water without getting turbid; it does not render chitinous structures brittle; and it can be used with advantage in the mounting of wet-fixed, Giemsa-stained slides.

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The Development and Affinities of the Pauropoda, based on a Study of *Pauropus silvaticus*

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PART I

With 9 Plates and 22 Text-figures

(*Editorial Note.*—A few references are made in Part I to figures that will be published in Part II. The list of references will be published at the end of Part II.)

Contents

Introductory

In the Pauropoda we have a group of very small and obscure myriapods in whose morphology, there is reason to suspect, some unusually primitive features are revealed. Their development may therefore be expected to throw light on some major problems connected with the evolution of the Myriapoda and their derivatives, the insects, and it is mainly with this end in view that the present work has been undertaken.

The gross anatomy of the adult animal is now fairly well known, having, since Lubbock's original work of 1868, been investigated by Schmidt (1895), Kenyon (1895), and Silvestri (1902). But there are many features of their structure, notably the all-important character of the mouth-appendages, upon which we are quite inadequately informed, but on which the assessment of affinities in large measure depends. In the following account, therefore, I have often found it necessary to prefix the embryological description of various organs with a brief, or at times even lengthy, statement on their adult morphology.

Accounts hitherto given of the development of *Pauropus* consist almost exclusively of a description of the external characters of various larval phases. The anamorphosis was discovered by Lubbock. Later writers—Latzel, Silvestri, Harrison, and others—have described more fully the individual larvae, though without adding much of significance to Lubbock's account. The eggs were discovered by Ryder as long ago as 1879. Harrison (1914) collected a large series of eggs of the Australian *Pauropus amicus*, but recorded nothing of their development beyond the fact that they appeared to undergo total cleavage. He made, however, the important observation that the young *Pauropus* left the egg, not as a larva, but as a motionless embryo, enclosed in an embryonic cuticle 'covered with long, tapering, cylindrical hair-like outgrowths'; and it is clear from his account that he had before him the 'pupoid'

phase. Beyond this, no observations on the development of *Pauropus* seem to have been recorded.

Bionomics and Life-History

Pauropus silvaticus inhabits the heavily timbered mountainous rain-forest country of Victoria. A taxonomic description of this species has been given in an earlier paper (Tiegs, 1943). It is a small, agile creature, and even when fully grown rarely exceeds 1·1 mm. in length. The animals may be found by turning over rotting logs, or by scraping away the leaves on the forest floor. But they also enter rotting timber, choosing by preference decaying trunks of tree-ferns (*Alsophila*, *Dicksonia*), and here they are sometimes present in great abundance. They favour damp localities, avoiding both dry and excessively wet places.

The associated microfauna comprises various species of Symphyla, Collembola, pseudoscorpions, mites, and small insect larvae; but from all these they are readily distinguished, even to the unaided eye, by their active mouse-like movements.

They are light-shy creatures, and, if dropped in an exposed place, commonly feign death. From the agility of their movements Latzel (1884) suspected that they were probably predaceous. *Pauropus silvaticus* I have, however, kept and bred in captivity for months in receptacles containing only fragments of decaying tree-fern; nor have I ever seen them, in the field, devouring the remains of other small creatures, though the reverse is sometimes the case, carnivorous mites and pseudoscorpions frequently taking toll of the larvae and even of the adults.

Oviposition takes place in early and middle summer, the eggs being laid singly in secluded clefts in the decaying vegetation in which the animals live. They are minute and spherical, measuring usually about 0·11 mm. in diameter, and under a hand-lens appear a smooth pearly white. In another species (*Pauropus amicus*) Harrison (1914) found that the eggs were laid in clumps, over which the female then mounted guard; *P. silvaticus* does not, however, display this maternal instinct, nor are the eggs ever laid other than singly.

Development within the egg occupies a minimum of 12–13 days; already on the tenth day a wide rent has appeared in the chorion, but it is not till 3 days later that the 'pupoid' phase is entered upon. The 'pupa' is still a quiescent stage, and in this condition the young animal remains for about 3 or 4 days longer. Eventually its cuticle splits along the mid-dorsal surface, and the larva emerges.

The first instar larva is a minute white creature, measuring not more than 0·25 mm. in length at the time of emergence. As Lubbock first observed, it presents already the main characters of the adult animal, but has only three pairs of legs. Moving at first sluggishly, it gains both in size and in strength, and soon acquires the agility of the adult. After about 3 weeks it moults, and discloses the second instar larva.

The latter, at the time of its first appearance, measures about 0·3 mm. in length; it possesses five pairs of legs (there is no larva with four pairs). The second larval stadium has a duration of about 4 weeks.

The third instar larva, which measures, at the time of its first appearance, about 0·5 mm. in length, has six pairs of legs. The duration of this stadium is about four weeks.

Two additional pairs of legs develop during the third larval stadium, the fourth instar larva having therefore eight pairs of legs. It measures 0·7–0·8 mm. in length; the duration of the stadium is about three weeks. At the end of this period the larva again moults, and so the adult stage with nine pairs of legs is attained. I have examined large numbers of exuviae collected from decaying wood in which the animals were abundant, but have failed to find any with more than eight pairs of legs; it would seem, then, that moulting ceases after the full complement of legs has developed.

The longevity of the animals is unknown; I have had some in captivity for nearly a year.

The duration of the larval period, viz. about 14 weeks, is much less than might have been expected from the statement of Lubbock that a first instar larva of *P. huxleyi*, kept in captivity by him for 6 weeks up to its death, failed to moult. My own observations have been made on only a few larvae in captivity. The duration assigned to the successive instars is approximate only; for the larvae are apt to disappear for days into clefts in the rotting timber where it is not possible to follow them without risk of crushing their excessively fragile bodies.

Methods

Owing to their unfortunate habit of laying their minute eggs singly in hidden clefts in the rotting timber in which they live, the collecting of an adequate supply of eggs is a very laborious task. About 200 eggs were obtained by carefully searching through infected logs brought into the laboratory; but I have also kept many animals in captivity, and from these have obtained a large supply of eggs. From the latter some approximately timed stages have been obtained; when ages are assigned in the text to particular embryos, they refer, in all cases, to laboratory-laid eggs at a temperature of 17°–20° C.

For fixing the eggs and pupae I have used Carl's fluid (acetic acid 2 parts, formalin 6 parts, alcohol 15 parts, water 30 parts). This fixative does not collapse the eggs, as does the more anhydrous Carnoy's fluid; it penetrates rapidly through the egg-membranes, which it is not necessary to puncture, about 15 minutes usually sufficing for fixation, after which the material is stored in 70 per cent. alcohol. Although the fixation is generally very good, it has the defect that it sometimes causes shrinkage of the already minute embryo. For larvae and adults this fixative is inadequate, since they are not wetted by it; Carnoy's fluid, on the other hand, gives excellent fixation, without the distortion that it produces in eggs.

As a stain for whole embryos and larvae I have used Auerbach's methyl green-acid fuchsin mixture. To admit the stain the egg-membranes or chitin of the larva are first punctured with a very finely ground needle. It is not necessary to unsheath the eggs for examination, for, after clearing, the embryo is visible in all detail through the delicate chorion.

Sections have, in all cases, been cut from celloidin-paraffin double-embedded material; for staining the sections I have used iron haematoxylin.

Observations on Embryonic Development

1. *The Egg* (*Structure, Oogenesis, and Fertilization*)

The egg is spherical and minute, measuring rarely more than 0·11 mm. in diameter, while at times eggs as small as 0·09 mm. are met with. The chorion is soft, and is covered with a multitude of minute spines, amongst which there is a single spine of much greater size (Text-fig. 1). In the great majority of eggs in which an embryo is present, this enlarged spine accurately marks the anterior pole of the egg; yet I have a few eggs in which this relation is quite clearly not observed.

The interior of the egg is occupied by yolk, the separate grains of which are supported within a fine framework of cytoplasm. A periplasm is not present, nor is there a vitelline membrane.

I am able to give only a meagre account of the meiosis, for I have had the usual difficulty of obtaining a sufficiently complete series of very early eggs. Such early stages as I have obtained were mostly got by frequent search in a receptacle into which some 80 adult animals had been placed. They lay, however, only at long intervals, and, moreover, only in the dark.

During the period of yolk-accumulation, and, indeed, up to a little before laying of the egg, the nucleus is in the germinal vesicle condition (fig. 2, Pl. 1). It measures about 25μ in diameter, displays several clumps of deeply chromatic substance, and a rather diffuse, weakly staining coagulum, within which only very doubtful indications of chromosomes are visible.

Shortly before the egg is laid the germinal vesicle gives way to a very characteristic phase, of which I have many examples, and which is shown in figs. 3 and 4, Pl. 1. The chromosomes have reappeared, and indeed with great clearness. They occur as 'tetrads', and of these there are thirteen. The diploid chromosome number is therefore twenty-six. They are not scattered at random, but lie uniformly in one plane. This is well seen in fig. 4, Pl. 1, which shows six of the thirteen bivalent chromosomes. Fig. 3 shows them in polar view. In favourable preparations spindle-fibres are faintly visible (fig. 4, Pl. 1). It is the metaphase of the first meiotic division.

I have no stage between this and the germinal vesicle. Presumably it has arisen by disruption of the latter, and the reappearance of the chromosomes within a central accumulation of cytoplasm; for neither in texture, nor in its further history, is the spherical body within which the chromosomes are lodged suggestive of nuclear material.

In the youngest-laid egg which I have obtained (it is probably not more than a few minutes old) this mass of cytoplasm has moved to the margin of the egg (fig. 5, Pl. 1), and within it the ensuing meiosis takes place. The chromosomes still lie, as tetrads, in one plane, but have begun to shrink a little. A reduction division now ensues, the chromosomes having shrunk still further into almost spherical masses. The anaphase of the reduction division is shown in fig. 6, Pl. 1; the division-plane, it will be observed, is not tangential, as usual, but radial. A polar view of this phase is seen in fig. 7, Pl. 1, and clearly shows the reduction in chromosome number.

Owing to lack of material I am unable to describe the process of second polar body formation. The polar body itself, however, and also the first, are clearly distinguishable up to about the time the male and female pro-nuclei have fused. Fig. 8, Pl. 1 shows them in a section that grazes the surface of the egg, and they are also indicated, in part, in figs. 9, 10, Pl. 1. From fig. 8, Pl. 1 it is evident that neither in the first nor in the second polar body do the chromosomes reassemble into a resting nucleus, and they do not even become enveloped in a nuclear membrane. The cytoplasm of the two is only very incompletely constricted, and, moreover, in neither polar body does it separate from the egg, but remains within its surface layer. The first polar body does not undergo further division.

Degeneration of the polar bodies usually sets in very early. In some eggs, even at the first cleavage, they can no longer be seen; yet in other cases they survive to the time when as many as eleven nuclei have appeared (figs. 40, 41, Pl. 4).

Fig. 9, Pl. 1 shows a section of an egg, of unknown age, in which the fully formed male and female pro-nuclei are for the first time distinguishable. The pro-nuclei lie within a central aggregation of cytoplasm, and within each the chromosomes are visible as delicate threads. The smaller of the two is probably the female nucleus, since it is nearer the polar body. I have not been able to recognize the sperm-head in any egg, and only very doubtful indication of a male pro-nucleus earlier than that just described.

The two pro-nuclei soon fuse to form the zygote nucleus (fig. 10, Pl. 1). The latter is a typical resting nucleus, within which chromosomes are not visible, the chromatin being concentrated partly within a small nucleolus, but otherwise scattered evenly in small grains throughout the nucleus.

2. *Segmentation of the Egg, and Formation of the Gastrula*

The large zygote nucleus now divides into a pair of cleavage-nuclei, which separate from one another, and move into opposite halves of the egg. Between the nuclei the remains of the spindle may persist for a time within the yolk (fig. 38, Pl. 4). In some eggs the cytoplasm which invests these nuclei is abundant, in others it is present in small amount only. From the delicate cytoplasmic reticulum in the interior of the egg a thin partition then forms, dividing the egg into its first two blastomeres, and these are roughly equal in size (fig. 11, Pl. 1). The groove demarcating

the blastomeres externally varies from one that is scarcely noticeable to a pronounced fissure.

I have only a single egg at the succeeding 4-cell stage (fig. 12, Pl. 1). In this particular egg the second cleavage has not been a complete equatorial cleavage, but the first two blastomeres have divided independently in planes at a right angle to one another and to the first cleavage plane. Whether the 4-cell stage is always arrived at in this way is, however, uncertain. A section through this egg is shown in fig. 39, Pl. 4.

I have not been able to recognize any further plan in the cleavage; for it is impossible to determine completely the orientation of the egg, and, moreover, partitions between the blastomeres may themselves be difficult at times to recognize. Cleavage, from now on, is markedly unsynchronized, both in respect to the incidence of nuclear division, and to the formation of cell-partitions. I have, for example, one egg in which, though there are eight nuclei present, only six blastomeres, of unequal size, have become demarcated; in another egg, with eleven nuclei, seven are undergoing division and the other four are resting.

Some eggs in successive stages of cleavage are shown in figs. 13–17, Pl. 1, and figs. 40–3, Pl. 4. Fig. 13, Pl. 1 depicts a 6-blastomere egg. An 8-blastomere egg is shown in fig. 14, Pl. 1, and a section through this egg in fig. 40, Pl. 4; the section shows, in the middle of the egg, the beginning of a segmentation cavity, where the innermost ends of the blastomeres have separated a little from one another. Fig. 41, Pl. 4 represents a section through an egg in which eleven nuclei are now present, of which some, but not all, are in mitosis; it will be noted that the plane of cleavage is radial, the spindle-axes lying tangentially. In this egg the segmentation cavity has not yet appeared. A more advanced egg, with 16 nuclei, but still only 11 blastomeres, is shown in fig. 15, Pl. 1. An egg with about 24 blastomeres is shown in fig. 16, Pl. 1, and a section through this egg in fig. 42, Pl. 4; the blastomeres are now typical 'yolk-pyramids', with the nuclei located in a clump of cytoplasm near the periphery, but still separated from the surface of the egg by several yolk-grains. There is now a conspicuous segmentation cavity. This simple cleavage, with the formation of 'yolk-pyramids' alone, ends at about the 40–5-cell stage. An egg at this phase of development (late blastula) is shown in fig. 17, Pl. 1, and a section through it in fig. 43, Pl. 4. The 'yolk-pyramids' have now grown narrower, and the segmentation cavity is even larger than in the foregoing egg. The nuclei are almost at the surface of the egg, with usually only a single layer of yolk-grains intervening.

By the time the number of cells has doubled, i.e. at about the 80-cell stage, a noteworthy change has taken place in the segmenting egg. An entire egg at this stage is shown in fig. 18, Pl. 1; a section through it in fig. 45, Pl. 4. The former segmentation cavity is now occupied by a clump of yolk, within which one or at most two large pale nuclei can be seen. As its further development shows, the central clump constitutes the endoderm; it is still very deficient in cytoplasm, and is recognizable as a distinct cell only by its large nucleus or

nuclei. The nuclei of the 'yolk-pyramids' have now mostly moved into the surface cytoplasm of the egg, and are flattened and much diminished in size. With the obliteration of the segmentation cavity a peripheral zone of large vacuoles appears in the yolk, and remains, for a time, a very conspicuous feature of the egg.

It is probable that the endoderm has arisen by the tangential, instead of radial, division of one or two 'yolk-pyramids'. I have not been able to observe the actual mitosis. I have, however, one egg in which the endoderm is seen in process of formation (fig. 44, Pl. 4); there are present 75 yolk-pyramids, each with its small nucleus located in the peripheral cytoplasm: the endoderm cell has partially obliterated the segmentation cavity and its outline is only vaguely defined, but its nucleus, already large, still lies towards the periphery of the egg. Whether the second endodermal nucleus arises in a similar way, or by division of the first, I have not been able to determine; the latter is probably the case, for the two nuclei are always found very close together.

There seems to be justification for regarding the stage, which is thus arrived at, as a gastrula (see further, section 5). This gastrula endures until about 160–200 cells have appeared in the outer layer. A drawing of the mature gastrula is shown in fig. 19, Pl. 1, a section through it in fig. 46, Pl. 4. In entire stained embryos the nuclei, further diminished in size, are now seen in great profusion at the surface, where yolk-grains are also still visible. The nuclei appear rather more crowded toward the future ventral pole of the egg. The peripheral cytoplasm within which they lie has begun to increase in concentration and thickness. Each nucleus, although lodged within the outer cytoplasm, is still the nucleus of a 'yolk-pyramid'. The latter cells have now acquired an almost columnar form, and their walls are becoming faint and difficult to distinguish, being quite imperceptible in surface views of whole eggs. In the central endoderm still not more than two large nuclei are present.

3. *Passage from the Gastrula to the Blastoderm Phase*

This takes place during the third day. At the periphery the cytoplasm continues to increase in quantity, and in some eggs, but not in all, attains even a considerable thickness. Within this peripheral cytoplasm the cleavage nuclei are almost all contained; the only exceptions are the nuclei of the central clump of endoderm and, in some eggs, a few enlarged nuclei of the 'dorsal organ' (see section 14 (vii)). Amongst the peripheral nuclei irregularly scattered mitoses are abundant.

The most noteworthy change in the interior of the egg is the breakdown of the partitions which have hitherto separated the 'yolk-pyramids', and therewith the yolk reverts to its originally unsegmented state. The wall which invests the central clump of endoderm can, however, often still be seen. Total cleavage of the egg thus gives way to a cleavage confined to the layer of surface cytoplasm. In good preparations cell-boundaries are easily distinguishable in this peripheral cytoplasm; but it is to be noted that there are no

cell-walls delimiting the latter from the intravitelline reticulum of protoplasm. The blastoderm, which thus develops, soon secretes a cuticle on its surface. This blastodermic cuticle is a very thin and perfectly smooth membrane, without any surface-sculpture. Unlike the chorion, it resists boiling with caustic soda.

A section through an early and still very thin-walled blastoderm is shown in fig. 47, Pl. 4; sagittal sections through two more advanced blastoderms in figs. 48, 49, Pl. 4. The appearance of the entire blastoderm is shown in fig. 20, Pl. 2. The surface aggregation of nuclei is evidently much more marked than in the gastrula, and the yolk has completely disappeared from the surface of the egg. The great change that has taken place in the surface epithelium will be seen by comparing figs. 50 and 51, Pl. 5, these drawings having been made from a mature gastrula and a mature blastoderm respectively, at identical magnification, and representing fragments of sections grazing along the surface of the egg. As the sections in figs. 47, 48, 49, Pl. 4, show, the internal wall of the blastoderm presents a very ragged appearance, for even in advanced blastoderms there are still no cell-walls delimiting it from the internal protoplasmic reticulum.

In actual appearance there is much variation between individual blastoderms. In some the epithelium remains quite thin, in others it becomes surprisingly thick. There is also frequently seen, even in young blastoderms, a precocious distinction into a thinner dorsal and a thicker ventral half; yet other, even mature, blastoderms may be of uniform thickness throughout. Eventually, however, in all cases a ventral thickening develops, in preparation for the impending formation of the germ-band.

In the central clump of endoderm there are still present not more than two nuclei; the cytoplasm has, however, begun to increase in quantity.

4. *Differentiation of the Germ-band from the Blastoderm, and the Formation of the Yolk-cells and Mesoderm*

By the fifth day the ventral thickening and attendant dorsal thinning-out of the blastoderm, referred to in the preceding section, have become much accentuated. Out of the lower thickened half the germ-band will develop; the dorsal thinner half will become the provisional body-wall. In whole preparations the two regions of the blastoderm are easily distinguishable, not only by differences in thickness, but by differences in spacing of nuclei, which are much sparser over the thin-walled half (fig. 21, Pl. 2).

Sections of the egg at this stage of development show the yolk-cells and mesoderm in process of formation. Already in late blastoderms isolated cells have separated from the epithelium, and are in process of invading the yolk (figs. 48, 49, Pl. 4); they soon become distinguishable by their large clear nuclei, with conspicuous nucleoli, and in this respect resemble the future yolk-cells, of which they are the forerunners, and out of which the fat-body will later develop. In blastoderms with ventral thickening these cells have increased in number, and a few may even be encountered deep in the yolk.

These 'yolk-cells' are not delimited by cell-walls, but are part of the intravitelline protoplasmic reticulum. The cells of the blastoderm, on the other hand, have now become sharply demarcated by cell-walls from this reticulum, only the few cells from which the 'dorsal organ' (see section 14 (vii)) will develop, remaining in continuity with it. The 'yolk-cells' arise at random from any part of the blastoderm; the mesoderm forms only from its ventral thickened half, and it is to this process of mesoderm formation, quite as much as to the local aggregation of blastoderm-cells, that ventral thickening is due.

Mesoderm formation does not, however, take place uniformly over the entire thickened ventral half, but is restricted to an area that corresponds roughly to the limits of the future germ-band; we find therefore, in sections that are taken 'horizontally' across the region of thickening (fig. 52, Pl. 5), that there is, on each side, a strip of intervening blastoderm where there is no mesoderm formation. In external views of whole embryos this zone without mesoderm is not distinguishable.

Where mesoderm formation is in progress, the cell-nuclei are piled several deep in the blastoderm (fig. 52, Pl. 5). Individual cells now separate from the latter, and gradually form into an irregular and broken layer of cells which remain adherent to the outer layer (fig. 53, Pl. 5). These mesoderm cells are readily distinguishable from the yolk-cells by their smaller and darker nuclei.

While the mesoderm is still in process of forming, the thickened epithelium on the lower half of the egg begins to acquire the contour of the germ-band. This is brought about by the lateral encroachment of the provisional body-wall on to the lower half of the egg, by thinning out of the mesoderm-free area; at the same time the zone of thickened epithelium spreads farther up over the anterior and posterior poles towards the mid-dorsal surface. A whole embryo at this stage of development is shown in fig. 22, Pl. 2, a sagittal section along it in fig. 54, Pl. 5. In this embryo stomodaeum formation is in progress. The stomodaeum is a small conical ingrowth of ectoderm, not yet showing a lumen, and therefore not visible in whole preparations. The yolk-cells are now scattered through the yolk. The mesoderm extends along most of the germ-band, and partially encircles the stomodaeum. Behind the latter it is heaped up, and this local accumulation of mesoderm is the source of the future pre-oral mesoderm. Farther to the rear the mesoderm forms a layer of irregularly scattered cells, with small cell-aggregations particularly along the lateral margins of the germ-band where the future somites will form.

The description of the mesoderm is continued in section 7.

5. *Gastrulation and the Formation of Germ-layers in Myriapods and Insects*

In the early development of myriapods and insects we are confronted with a type of ontogeny whose meaning has been the subject of much speculation. What is the significance of the blastoderm? Is there a gastrula? Does development proceed in conformity with germ-layer principles? *Pauropus* shares with other myriapods and with insects some of the features

of their specialized development; but the presence of a thinly veiled gastrula is unexpected. Its existence offers new scope for discussing the above questions.

In all species of *Peripatus* whose development has been examined, a gastrula can be recognized. In *P. capensis* it is an epibolic gastrula, in which both archenteron and blastopore are present (Sedgwick, 1885). The two other yolk-deficient species whose development is known—*P. edwardsii* (Kennel, 1884), *P. imthurni* (Sclater, 1888)—seem to lack a blastopore, and in the former even the appearance of the archenteron is delayed. Special significance attaches to the species with yolk-laden eggs. Of these the early development of two has been examined—*P. novae-zelandiae* (Sheldon, 1887–8), *P. weldoni* (Evans, 1901); in both there is a long ventral blastopore, from which endoderm grows inwards around the yolk, but an archenteron does not form. In all the species the mesoderm is found to arise from the neighbourhood of the blastopore. The formation of an embryo then proceeds without the intervention of a blastoderm phase.

In *Pauropus* also, with from 80 to 200 cells present, a stage is attained to which the status of a gastrula must be conceded; for it consists of a central clump of endoderm, out of which mid-gut epithelium will develop, and an outer layer of cells which may justly be considered as ectoderm. It is true that neither blastopore nor archenteron are present; but neither of these is a constant feature of the gastrula of *Peripatus*. The gastrula of *Pauropus* is, nevertheless, a much-modified gastrula; its ectodermal cells are fully as rich in yolk as are the endodermal cells, and their nuclei, in anticipation of the formation of the blastoderm, are already located at the surface. Eventually by the breakdown of the walls of the ectoderm cells and the accumulation of a surface layer of cytoplasm the blastoderm phase is entered upon, and cleavage thereafter becomes a purely superficial cleavage on the outside of an unorganized mass of yolk. The blastoderm then becomes demarcated into a germ-band and a provisional body-wall. The mesoderm forms by separation of cells from the germ-band, and not from a blastoporal area, as in *Peripatus*. It is evident that the blastoderm must be a post-gastrula phase, and is not represented in the development of *Peripatus* (in the heavily yolked eggs of *P. novae-zelandiae* Sheldon refers to a 'blastoderm'; this is, however, a pre-gastrula phase, and is not comparable with the post-gastrula blastoderm of *Pauropus*).

In no insect and in no myriapod with the possible exception of *Scolopendra* is there such clear evidence of the presence of a gastrula as in *Pauropus*, for in one way or another the egg passes directly into the blastoderm phase. The egg of Symphyla shows, at the blastoderm stage, a remarkable resemblance to that of *Pauropus*, for the 'yolk-cells' enclosed by the blastoderm are not completely specialized vitellophages, but survive, for the greater part, to form fat-body and mid-gut epithelium; the mesoderm also arises, as in *Pauropus*, by separation of cells from the germ-band. Judging by Heathcote's (1886) fragmentary description of the development of *Julus terrestris*, the yolk-cells

of Diplopoda are, like those of *Pauropus* and Symphyla, partly used in the formation of adult tissues, for the fat-body seems to arise from them. Heathcote derived the mid-gut also from a central core of yolk-cells, but all other authors (Metchnikoff, 1874; Cholodkowsky, 1895; Lignau, 1911; Pflugfelder, 1932) are agreed that it takes its origin from the germ-band itself, either in association with the stomodaeum alone, or with the latter and the proctodaeum, the two Anlagen then growing towards each other through the yolk. In Diplopoda the mesoderm arises by separation of cells from the germ-band.

In *Scolopendra* alone among myriapods do we find a form of development which is strangely reminiscent of that found in the heavily yolked species of *Peripatus*. According to Heymons' (1901) account, most of the cleavage-nuclei move from the middle of the yolk towards the periphery, where the blastoderm phase then ensues, only a few of the nuclei remaining behind in the yolk as nuclei of vitellophages. From a small area in the blastoderm (cumulus primitivus) more vitellophages then enter the yolk. This is followed by the separation of endoderm cells over the lower half of the blastoderm, these cells being applied, as mid-gut Anlage, to the outer surface of the yolk. Most of the mesoderm arises by proliferation from the cumulus primitivus of two forwardly moving bands of cells, and here we are strongly reminded of the formation of the mesoderm in *Peripatus* from a blastopore. It would seem that the 'blastoderm' of *Scolopendra* is a blastula, comparable with the 'blastoderm' of some heavily yolked species of *Peripatus*, and that it is not the equivalent of the post-gastrula blastoderm of *Pauropus*.

We turn now to the thorny question of the interpretation of the blastoderm of insect embryos. The conviction that a gastrula, with its ancestral germ-layers, must at all costs be revealed in the development of these arthropods seems to have introduced little but confusion into the subject.

Haeckel (1877) regarded the blastoderm as a modified blastula. Supposedly basing his interpretation on Kowalewsky's description of the development of *Hydrophilus*, Haeckel identified the gastrula with the immediately following stage in which the ventral groove invaginated. Yet Kowalewsky had observed that the invaginated 'lower layer' was not endoderm, nor its cavity an archenteron.

Balfour (1880) saw the error of this interpretation. Though accepting the view, then prevalent, that the mid-gut arose from yolk-cells, he denied the existence of a gastrula in the development of insects on the ground that the formation of the yolk-cells could not be reduced to an invagination process.

In a later work Kowalewsky (1886) reintroduced Haeckel's gastrula, though in a modified form. He had observed the formation of the mid-gut, not from yolk-cells, but from the anterior and posterior 'endoderm rudiments' which themselves form the anterior and posterior tips of the 'lower layer' that invaginates along the ventral groove. For him the invaginating groove was an elongate blastopore, with the endoderm confined to its ends, and with a long

strip of mesoderm intervening. This view has found favour with many writers, the more so since the divided blastopore of *Peripatus* seemed to afford a possible clue to the origin of the elongate 'blastopore' of insects.

Kowalewsky's gastrula met its first important criticism in Heymons' great work on the development of Dermaptera and Orthoptera (1895). In these primitive insects the mid-gut was found to arise by ingrowth of cells from the stomodaeum and proctodaeum. In some species the ventral groove was not even present, but the mesoderm arose by separation of cells from the germ-band. This latter type of mesoderm formation is common to all myriapods hitherto examined except *Scolopendra*, as well as to the Collembola, *Lepisma*, *Campodea*, *Eutermes*, and many Orthoptera. The ventral groove, far from being a modified blastopore, is thus found to have originated among the Insecta themselves.

Heymons' observation that the mid-gut epithelium developed from stomodaeum and proctodaeum was in such obvious conflict with current notions of morphogenesis, based on an acceptance of Haeckel's 'Gastraea theory', that it encountered much opposition—Nusbaum and Fulinsky (1906), Hirschler (1909), MacBride (1914), and others. Yet it has been repeatedly confirmed, and especially by more recent writers, for insects of different orders—*Diacrisia* (Lepidoptera), Johannsen (1929); *Calandra* (Coleoptera), Mansour (1927), Tiegs and Murray (1938); *Locusta*, Roonwal (1936–7); and *Pteronarcys* (Perlaria), Miller (1940)—and seems to hold also for some Diplopoda.

Applying germ-layer terminology, Heymons concluded that the mid-gut in Orthoptera was 'of ectodermal nature'. It is not necessary to consider the validity of this proposition; it will suffice to agree that it cannot be endodermal. For the term 'endoderm' must be restricted to the mid-gut Anlage when this forms the inner layer to a gastrula, unless it is to become a superfluous alternative to 'mid-gut-epithelium' in general. Consistently with germ-layer principles, the need now arose for identifying endoderm in the embryo. In this dilemma Heymons, and many others following him, have turned to the yolk-cells. The development of *Campodea* and *Lepisma* seemed to lend countenance to this view, for in these insects the mid-gut arises from cells which, migrating from the blastoderm into the yolk, assumed control over the yolk itself.

In *Pauropus* and *Hanseniella* (Symphyla) also the mid-gut is derived from yolk-cells; yet it is to be observed that in these myriapods most of the 'yolk-cells' are used, not in the formation of mid-gut epithelium, but of fat-body, a tissue which in insects is of mesodermal origin. In *Scolopendra*, as Heymons has shown, true yolk-cells (vitellophages) and endoderm are simultaneously present; had the mid-gut in this myriapod developed instead from stomodaeum and proctodaeum, as it does in many insects, then assuredly the vitellophages would have been invoked to uphold the germ-layer principle.

The validity of identifying yolk-cells, or any other cells, with endoderm, when these do not give origin to the mid-gut, involves the difficult theoretical problem of applying the principle of homology to an undifferentiated region

within an embryo. The concept of homology has emerged from a comparison of organs. Spemann (1915), in a lucid discussion on the propriety of applying this concept to unorganized regions of an embryo, writes: 'Homologizing is only possible after the formation of Anlagen, i.e. at a developmental period when the individual parts of the germ have become differentiated, if not in their outward appearance, at least in their developmental tendency.' To homologize yolk-cells with endoderm therefore implies a belief that they are the vestige of a discarded mid-gut, and that the 'ectodermal' mid-gut is a completely new organ.[1] Such are the difficulties, and indeed absurdities, which follow any attempt to explain away discrepancies in an outworn theory of development that did not contemplate such facts.

In the development of *Pauropus* we now seem to have a clue to the interpretation of the blastoderm phase; it is a stage which succeeds a transitory gastrula, and is not, therefore, comparable with the 'blastoderm' of a heavily yolked *Peripatus* egg, which is a modified blastula. In other myriapods and in insects the gastrula has become superseded altogether, and the segmenting egg then passes directly into the blastoderm phase. It is a distinctive feature of these ontogenies that the mesoderm develops by separation of cells, either diffusely, or bilaterally, along the germ-band, or by the more specialized process of invagination along a ventral groove, and not, as in *Peripatus*, from a blastoporal region ('primitive streak'); in *Scolopendra* alone does a more primitive mode of mesoderm formation, by cell-proliferation from a 'cumulus primitivus' (primitive streak), seem to have largely survived.

The function of the blastoderm is evidently to eliminate the impeding action of yolk on morphogenetic processes in the egg. In Pauropoda, Symphyla, many Collembola and Diplopoda, the adaptation to yolk-accumulation is imperfect, for it is certainly surprising to observe the interior of the egg reverting to an unsegmented condition after the initial phase of apparently futile total cleavage. In *Pauropus* these early cleavages even permit the formation of a gastrula. In all these primitive groups, moreover, yolk-laden cells in the interior of the egg, after playing an initial role as vitellophages, become organized into specific tissues of the adult. In insects (except Collembola and a few parasitic forms) there is no segmentation of the egg attending cleavage of the nuclei, and the egg passes directly into the blastoderm condition. Yolk-cells (except in *Lepisma* and *Campodea*) are now purely vitellophages, and the tissues which develop out of yolk-cells in the more primitive groups are now organized from other sources. It is evident that as this specialized type of development has evolved, all trace of a gastrula, or of a gastrulation process, as still exemplified in *Pauropus*, has been lost.

Vertebrate embryos, confronted with much greater problems of yolk-accumulation, have, like those of the heavily yolked species of *Peripatus*, preserved to a surprising degree the primitive type of development of their ancestors; for the early developmental processes of vertebrates, however

[1] One writer (Lecaillon 1898) has even seriously proposed thus to avoid conflict with the germ-layer theory.

much obscured, can still be reduced to a gastrulation process, and do not show such remarkable departures from the primitive as the developing insect reveals.

6. *Development of the External Characters of the Embryo*

(i) *Early Development of the Germ-band, and the Formation of Appendages.* By about the end of the fifth day the head-lobes have appeared (fig. 23, Pl. 2). In the region of these lobes the germ-band is at its greatest width. The lobes are not yet very pronounced; they are, however, distinctly paired, for there is a deep indentation between them at the anterior tip of the germ-band. By this time a small elongate stomodaeal orifice has arisen, and the proctodaeum is now also in course of formation.

During the sixth day the antennae begin to form. In entire embryos they may be seen as a pair of just perceptible backwardly directed elevations on the head-lobes, a little behind the stomodaeum, their outline being most readily distinguishable along their inferior margin (fig. 24 A and B, Pl. 2). There are no intersegmental grooves delimiting the antennary segment, and, indeed, throughout the length of the germ-band such grooves do not appear until the embryo is in an advanced state of development.

By the seventh day the embryo has assumed the form shown in fig. 25 A and B, Pl. 2. The stomodaeal opening is still conspicuous. The head-lobes have now much enlarged (cf. the relative position of the stomodaeum in figs. 23, 24 B, and 25, B, Pl. 2), and show the orifices of a pair of deep invaginations, which are the Anlagen of the posterior lobes of the protocerebral ganglia. The antennae are considerably enlarged and more sharply defined; their bases now lie on about a level with the stomodaeum. Behind the antennae evidence of two new segments has appeared, namely the pre-mandibular and mandibular, but neither is demarcated by transverse grooves. The former occupies a large area of the germ-band behind the antennae, but at no time does it possess even a rudiment of appendages. The mandibular segment, on the other hand, is made evident externally by its pair of large mandibles; they are still little more than gentle elevations of the surface of the germ-band, the alignment of nuclei around their margin accentuating their outline.

Before the end of the seventh day several additional segments have become defined. An embryo at this stage of development is shown in fig. 26 A and B, Pl. 2. The stomodaeal opening has now become a wide transverse cleft. The antennae are much more distinct. The mandibles have also become more sharply defined, and the first outlines of the maxillae are distinguishable behind them. It is to be observed that both these appendages are, from the first, directed medially. The first leg-bearing (second abdominal) segment is now also becoming recognizable by the presence of its appendages, which arise as gentle protuberances from the germ-band, farther from the mid-line than the gnathal appendages, and at first much smaller than these. Between the maxillary and first leg-bearing segment is the collum (post-maxillary) segment, but it does not bear the Anlagen of any appendages; it does not become part of the head, but forms the first of the abdominal segments. By this time

certain 'ventral organs' (cf. section 13) have appeared, and these afford a useful guide to the subsequent displacement of the head-segments; they do not protrude beyond the surface, but are recognizable in stained whole embryos by the peculiar radiating orientation of their nuclei. They are already present in the pre-mandibular, mandibular, and maxillary segments (fig. 26 B, Pl. 2).

A rather more advanced embryo, aged about 8 days, is shown in fig. 27 A and B, Pl. 2. The first legs have considerably enlarged, and the second have also appeared. Behind these a pair of just perceptible swellings on the germ-band indicates the formation of the third legs. On the head the orifices of the invaginating posterior lobes of the protocerebral ganglia, which have hitherto been conspicuous, are no longer present. The maxillae are now sharply defined. The only other noteworthy change concerns the beginning of formation of the pre-oral cavity ('mouth-cavity'), a description of which is given below (see Development of Head).

I have already referred to the absence of any intersegmental grooves whatever in the early embryo. By the eighth day, however, a rather vague indication of some of the segments has appeared, in the form of slight encroachments from the margin of the germ-band on to the provisional body-wall, and between them perceptible grooves are appearing. These grooves, it must be stressed, are confined to the lateral margin of the germ-band, and do not extend on to its lower surface. They are visible in the embryo shown in fig. 27 A, Pl. 2, those that define the collum and second abdominal (first leg-bearing) segments being the first to appear.

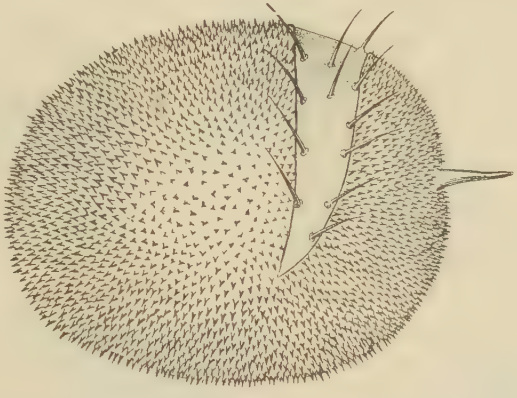
Thus far the distinction between the germ-band and the thin provisional body-wall has been preserved. In the latter, however, mitoses are not infrequently seen, and there is not that paucity of cells that we find commonly in the provisional body-wall of other myriapods and insects. In some embryos the nuclei are even closely crowded (e.g. fig. 26 A, Pl. 2), but there is much variation in this respect. In all cases, however, it is a thin membrane, and the yolk is always easily visible through it.

(ii) *The Advanced Embryo.* During the eighth day the first embryonic cuticle begins to form. It is rather thicker than the blastodermic cuticle. Its most striking feature is a band of setae, which lies transversely across the head. The individual setae that compose the band are relatively long, stout, and sharp, and are disposed in three transverse rows, there being eleven in the first, ten in the last, and a short row of four between them. In 9-day embryos these setae can readily be seen through the transparent egg-membranes lying against the surface of the embryo. During the tenth day they become erect, and thereby tear a wide transverse rent in the overlying blastodermic cuticle and chorion, through which they now protrude (Text-fig. 1). Advanced eggs can always readily be identified by these protruding setae; the egg is, moreover, no longer spherical, but has begun to lengthen.

A few days before the embryo is due to emerge from the egg a second embryonic cuticle is formed. This is the 'pupoid' cuticle. It also is adorned with setae; but unlike those of the first cuticle, they are mostly very long,

curved, and delicate, and are distributed over much of its dorsal and lateral surfaces. As the second cuticle develops, the tear in the egg-membranes slowly enlarges. On about the twelfth or thirteenth day it widens, a rent then appearing also in the embryonic cuticle, and so the pupa is slowly liberated (Text-fig. 3). The discarded chorion, blastodermic, and embryonic cuticles remain adhering to its under surface.

In describing the changes which meantime have taken place in the external form of the embryo, it will be convenient to consider the abdomen and head separately.



TEXT-FIG. 1. Egg, showing initial rupture of chorion; cutting setae of embryonic cuticle protruding. Anterior end to right.

A. *The Abdomen.* During the ninth day the formation of the definitive body-wall is in active progress (fig. 28 A, Pl. 3). All along the margin of the germ-band cells are beginning to spread upward, and the intersegmental lines delimiting the collum and second abdominal segment are now quite clear, owing, in a measure, to the fusiform character of their cells and of their nuclei.

In the advanced embryo shown in fig. 29 A, Pl. 3 (aged about 10 days), the body-wall is almost complete. The intersegmental lines have now deepened into grooves, the groove behind the first leg being an exceptionally deep cleft. The grooves completely encircle the embryo, spreading now even on to its ventral surface; it is, however, on the dorsal surface that they attain their greatest depth. This dorsal accentuation of the clefts is evidently a consequence of the transverse folding to which the epidermis is there subjected, for the embryo is still in a somewhat dorsally flexed condition.

The segments which have thus become demarcated are the collum and second to fourth abdominal segments. The collum segment is markedly wedge-shaped, its tergal portion being reduced to a narrow strip of epidermis. Some time before hatching, its sternal wall enlarges almost to the size of the segment that succeeds it (cf. figs. 29 B and 30 A, Pl. 3). The fourth abdominal segment, which bears the third pair of legs, is also a wedge-shaped segment,

for the intersegmental line that delimits it behind merges, toward the dorsal surface of the embryo, with the intersegmental line behind the preceding segment (figs. 29 A, 31, Pl. 3). The pleural areas of the collum and three leg-bearing segments are thin, and through them the yolk is still readily seen. These areas remain permanently thin, for there are no muscles attached to them.

It is in these advanced embryos that we see the first indication of the intersegmental line that delimits the fifth abdominal from the anal segment. If the embryo is turned on its end, so that it can be viewed from behind, the limits of the new segments are already seen clearly outlined by a ring of fusiform cells, even though an actual groove is not yet present. Near the middle of the last segment is the anus (fig. 31, Pl. 3).

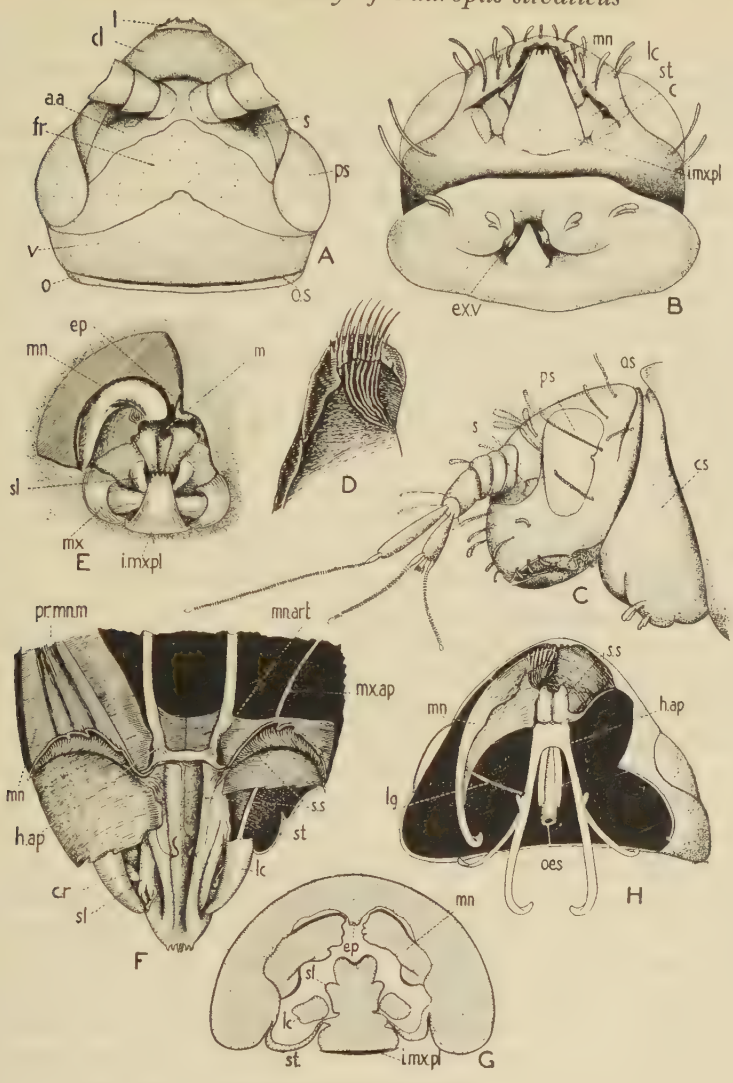
On the lateral part of the tergal wall of the fifth and third abdominal segments there may be seen, in all embryos after the tenth day, a pair of small but conspicuous patches of rather deeply staining cells; they are the developing sensory setae (trichobothria); figs. 29A, 31, Pl. 3.

On the ninth day the legs begin to elongate more actively. Unlike the mouth-appendages, they are, from the beginning, outwardly directed. They do not show any localized growing zone, for mitoses appear at random along their length. They soon become bent on themselves. Thereafter the development of the first pair, hitherto much the largest, is retarded, the second and third even outstripping the first a little. In the advanced embryo the legs become narrower, especially towards their tips, where they bend well under the embryo. Only at about the time of liberation of the pupa does the first sign of their segments appear.

On the collum segment legs are absent. On the floor of this segment there are present a pair of large swellings, each bearing a minute medially directed conical papilla (Text-figs. 2 B, 24 B). Following Latzel (1884) it is customary to regard the latter as the vestigial appendages of the collum segment. The swellings on the floor of the segment which bear these problematical organs do not appear till about the tenth day, and show no obvious resemblance to limb-Anlagen (fig. 30 A, Pl. 3). They are referred to again in section 14 (v).

B. *The Head.* (*a*) Morphology of adult head (Text-fig. 2). The descriptions of the head-capsule that have passed into current authoritative works on the Pauropoda are inadequate, and incorrect on points of importance; for, from its minuteness and the complexity of its structure, this is a most difficult part to examine. The following account is given to facilitate the embryological description which follows; but a knowledge of the structure of the head is also of much importance for the assessment of affinities.

The head, which is carried in a depressed (hypognathous) attitude, appears somewhat triangular when viewed from above. Four sutures run across its dorsal surface, which is thereby divided into five areas (Text-fig. 2 A). I shall speak of these as the clypeus, antennal area, frons, vertex, and occiput; the terms have been chosen for convenience, and no homology with any similarly named parts of an insect head is implied. The line between clypeus and



TEXT-FIG. 2. The Head.

A. Dorsal view; setae not drawn. B. Ventral view; collum segment included in drawing; left maxilla has been drawn out from under intermaxillary plate. C. Lateral view; collum segment, and part of succeeding segment, included in drawing. D. Tip of right mandible, viewed from below. E. View into pre-oral cavity (diagrammatic). F. View from above, to show floor of pre-oral cavity; part of the right mandible and most of the left removed; cavity of left maxilla partly exposed; part of interior of head-capsule also shown. G. Transsection through head, taken at level of bases of superlinguae. H. Ventral view of head, showing suspensory sclerite, hypopharyngeal apophyses, and right mandible.

Lettering. *a.a* antennal area; *c* cardo of maxilla; *cl* clypeus; *c.r* chitinous ridge against which maxilla rests; *c.s* collum segment; *ep* epipharynx; *ex.v* exsertile vesicle; *fr* frons; *h.ap* hypopharyngeal apophysis; *i.mx.pl* intermaxillary plate; *l* 'labrum'; *lc* lacinia of maxilla; *lg* fibrous ligament between mandibular apodeme and hypopharyngeal apophysis; *m* mouth; *mn* mandible; *mn.art* articulation of mandible with hypopharyngeal apophysis; *mx* maxilla; *mx.ap* maxillary apodeme; *o* occiput; *oes* oesophagus; *o.s* occipital suture; *pr.mn.m* protractor muscle of mandible; *ps* pseudoculus; *s* sclerite for attachment of dorsolateral longitudinal muscle of head; *sl* superlingua; *s.s* suspensorial sclerite; *st* stipes of maxilla; *v* vertex.

antennal area is a deep recurving fissure; that between the vertex and occiput (occipital suture) forms a strong phragma for the attachment of tergal muscles. The other two sutural lines are merely thin seams in the dorsal chitin, and are easily overlooked. The occipital suture appears, from its development, to be the mandibulo-maxillary intersegmental line, the short occiput to the rear of it being the reduced tergal wall of the maxillary segment; whether the other sutures have any segmental significance cannot be determined. The chitin of the clypeus becomes thinned out, at its tip, to form a very inconspicuous 'labral lobe', fringed with tiny curved spines, and demarcated by a groove from the clypeus. Along its margins the latter is, as Silvestri (1902) has already shown, folded in beneath the mandibles, which are therefore shut in except at their tips (Text-fig. 2 B).

On the sides of the head are the large bulging 'pseudoculi'. These are described in section 14 (iii).

Immediately behind the bases of the antennae, in the position occupied by the post-antennary organs of Tömösvary in Symphyla, are a pair of small rectangular sclerites (Text-fig. 2 A, C); they do not, however, represent such an organ, but are thickenings of the chitin, to receive the insertion of the dorso-lateral muscles of the head (see section 15).

The bifurcated antennae (Text-fig. 2 C) arise close together on the antennal area; adequate descriptions of these remarkably specialized appendages have already been given by Lubbock, Latzel, Kenyon, Remy, Silvestri, and others.

The examination of the mandibles presents considerable difficulty, for the conformation of their chitin is complex, and they are, moreover, closed in by the inturned folds of the clypeus. They consist each of an unsegmented piece of chitin, whose base is prolonged into an apodeme, a long, curved blade of chitin, which extends far back into the cavity of the head (Text-fig. 2 H). The apodemes are attached each by two fibrous ligaments, a median to the hypopharyngeal apophysis (Text-figs. 2 H, and 12), and a lateral to the wall of the head just to the rear of the pseudoculus (Text-fig. 21 A). Each mandible is articulated with the hypopharyngeal apophysis (Text-fig. 2 F, H). Only the anterior quarter of the mandible is free to operate within the pre-oral cavity, and here it displays a most unusual structure: on its superior surface the chitin is thick and firm, but on its inferior surface it is thin and has the form of a shallow groove. Along this groove run fine ridges of chitin, about seven in number, and these are prolonged beyond its tip to form a comb of seven delicate curved blades, beyond which is a second row of smaller blades (Text-fig. 2 D, H). The two grooved surfaces of the mandible co-operate with an epipharyngeal ridge to form the upper half of a tube, the lower half of which is completed by the grooved floor of the pre-oral cavity, against which a flange from the mandibles fits; and the whole structure is evidently a contrivance for drawing into the mouth semifluid food scraped away by the fine terminal blades of the mandibles. As other authors have already observed, the intestinal content has a fluid consistency. Nevertheless, animal remains are occasionally met with. For their possible origin see section 9 (*a*).

Below the mandibles is a single pair of maxillae, and between them the intermaxillary plate (Text-fig. 2 B). The latter is a simple triangular piece of chitin, bearing at its tip several short, blunt spines. The maxillae are composed of two parts, distinguishable as stipes and lacinia. There is no galea or palp. The lacinia is an elongate tapering piece of chitin, with a slender apodeme that extends far into the head, where it becomes attached to one of the ligaments of the mandibular apodeme. The lacinia is partly covered, from below, by the intermaxillary plate, and rubs against an inturned chitinous process from the latter (Text-fig. 2 F). The stipes is a short, semi-cylindrical sclerite devoid, as usual, of an inner wall, and forming therefore part of the head-wall itself. Behind the latter is another, and smaller, sclerite, the equivalent, it would seem, of the cardo; but, unlike the other two parts of the maxilla, it has no muscle-attachments and should therefore probably not be reckoned as part of the appendage itself, but rather as a 'pleurite'.

The intermaxillary plate, which is the sternite of the maxillary segment, does not itself constitute the floor of the pre-oral cavity, the latter being formed mainly out of the inturned sternum of the mandibular segment, the whole protruding structure being a kind of 'lower lip'. Along it runs the gradually widening and deepening groove above alluded to, which leads behind into the mouth (Text-fig. 2 E, F). To the sides are a pair of appendage-like structures, ending in several minute teeth, and already referred to by Silvestri (1902) as the galeae of the intermaxillary plate; actually, however, they arise from within the pre-oral cavity (Text-fig. 2 G), with the floor of which they are flexibly articulated, but they do not seem to be provided with muscles. They are a product of the mandibular segment, and seem therefore to be the equivalent of the superlinguae.

A pair of unusually complex suspensorial sclerites for the attachment of the hypopharyngeal apophyses is present (Text-fig. 2 F, H). They occupy part of the hinder wall of the pre-oral cavity, above the bases of the mandibles, where there are attached to them the protractor muscles of these appendages (see section 15). From here they extend down below the mouth, and then bend forward to form a support for the floor of the pre-oral cavity. They are not associated with the superlinguae.

The hypopharyngeal apophyses (Text-fig. 2H) are a pair of complex chitinous structures, from which many of the sternal muscles of the appendages take origin. (Ferris (1942) has justly criticized the term 'hypopharyngeal apophyses' which I have retained here in deference to its use in recent standard works on insect morphology (Snodgrass, Weber). The apophyses do not arise from the hypopharynx, even when that term is used, in the wider sense, to include the superlinguae.) Immediately behind their point of attachment to the suspensorial sclerites they form a chitinous ring round the oesophagus, from which two sheaths extend back some distance along the latter. The principal arms of the apophyses pass back into the collum segment, where they bend round the tritocerebral ganglion to provide attachment for the large dilator muscles of the oesophagus (Text-fig. 12). In addition

there is present a pair of smaller arms, which bend upwards and become attached by fibrous tissue to the occipital suture; these act as braces for the whole structure, and there are no muscles attached to them. The development of the apophyses is described in section 14 (vi).

(*b*) Development of the head. The principal theoretical problem in the developing head is the determination of its segments, and the identification of these in the completed head-capsule. Most morphologists agree that the procephalon has arisen by the welding together of an acron (prostomium) and three segments, viz.: the pre-antennary, antennary, and pre-mandibular. The principal evidence for this is provided by Heymons' (1901) study on *Scolopendra*, in the embryo of which the constituent segments of the procephalon are identifiable, to an unusually complete degree, by their ganglia, mesodermal somites, and intersegmental grooves. The hope that so primitive a myriapod as *Pauropus* might yield still more complete evidence on this point has not been fulfilled, for intersegmental lines are here markedly suppressed throughout the whole length of the germ-band. The chief external guide to the segments must therefore be the appendages and the 'ventral organs'. The latter are well developed on the antennary and pre-mandibular segments, those of the pre-antennary segment being diminutive; appendages occur on the antennary segment alone. The pre-antennary segment and acron are not externally distinguishable.

In the gnathocephalon two segments only are present, the mandibular and maxillary; there is no second maxillary segment. Each has a pair of large appendages and a pair of 'ventral organs'. An intersegmental line demarcates these two segments from one another, but, like the other intersegmental lines, it does not appear until late in the development of the embryo.

By the sixth day the antennae have begun to form. They are, at first, merely gentle swellings of the epidermis, and, as usual in myriapods and insects, lie post-orally (fig. 24 B, Pl. 2). By the seventh day they are more distinct, and have moved up almost into line with the stomodaeum; the mandibles have now also begun to form, and the pre-mandibular segment is distinguishable (fig. 25 B, Pl. 2). In the late 7-day embryo, as shown in fig. 26 B, Pl. 2, the bases of the antennae lie on a level with the stomodaeum, which is now a wide slit; the maxillae are beginning to form, and the pre-mandibular, mandibular, and maxillary 'ventral organs' are distinguishable. The impression obtained from this series of embryos is that a post-oral antennary segment is in process of curving forward round the stomodaeal opening.

During the eighth day the pre-oral cavity begins to form. The head is still very large and occupies most of the anterior half of the egg (fig. 27 A, Pl. 2). The mandibles have moved nearer to the mouth, and the pre-mandibular segment therefore appears of diminished size. This is due to the fact that the median sternal portion of the segment is beginning to invaginate in the direction of the former stomodaeal opening, pushing the latter more deeply into the head. The new cavity which is thus forming is the pre-oral cavity, and its floor consists of pre-mandibular epidermis (fig. 27 B, Pl. 2). At its corners

are situated the 'ventral organs' of the pre-mandibular segment. If the head is turned on its side and examined in optical section the pre-mandibular (tritocerebral) ganglia, hitherto post-oral in position, are now seen to the side of the stomodaeum. It is therefore clear that the pre-mandibular segment, like the antennary, has now also begun to curve forward from behind the original stomodaeal opening.

In the 9-day embryo shown in fig. 28 A and B, Pl. 3, the antennae have moved into a pre-oral position, and the mandibles now lie in line with the pre-oral cavity. The median sternal portion of the pre-mandibular epidermis has completely disappeared from the surface; but if the head is turned on its side, it can be seen, in optical section, forming the floor of the pre-oral cavity. Comparison with fig. 27 B, Pl. 2, shows, at the same time, that the more lateral part of the pre-mandibular epidermis has moved into a pre-oral position, and occupies an area, with unidentifiable outline, median to the base of each antenna; this epidermis later plays a large part in the formation of the clypeus, and it is therefore evident that, as in Symphyla, this part of the head-capsule must be largely of pre-mandibular origin. (Both in insects and myriapods the innervation of the clypeus and labrum from the tritocerebrum has long been known (Saint Remy, 1887; Viallanes, 1887; Holmgren, 1916), and there can be little doubt of an extensive participation of the pre-mandibular segment in the formation of these parts in other myriapods and insects.) The sternum of the mandibular segment is now a conspicuous triangular structure forming a lower lip to the pre-oral cavity (fig. 28 B, Pl. 3); already in the earlier embryo shown in fig 27 B, Pl. 2, this structure is recognizable by the arrangement of its nuclei.

In the 10-day embryo shown in fig. 29 A and B, Pl. 3, the antennae have grown longer and more slender, and their bases have begun to approach one another on the anterior wall of the head. There can no longer be any doubt as to the reality of the displacement and deformation to which the remarkable antennary segment is subjected. The clypeus has now started to fold down over the bases of the mandibles, and this has the effect of greatly widening the pre-oral cavity and of enclosing the mandibles within it. The mandibular sternum still protrudes as a kind of lower lip to the pre-oral cavity. The head has, by this time, already begun to diminish in relative size (cf. fig. 27 A, Pl. 2, and fig. 29 A, Pl. 3).

Enclosure of the mandibles within the pre-oral cavity is not complete until shortly before the embryo leaves the egg as a 'pupa'. An embryo at this stage of development is shown in fig. 30 A and B, Pl. 3. The maxillae are now conical in form, and protrude towards the tip of the clypeus. Between the maxillae is the intermaxillary plate. The mandibular sternum does not form a part of it, but it is derived entirely from the maxillary sternum. From the structure of the head in the earlier embryos shown in figs. 28 B and 29 B, Pl. 3, the participation of the mandibular sternum in its formation might have been expected; there is, however, no evidence for this, and indeed, if the embryo be turned on end to admit a view into the pre-oral cavity, the inturned mandibular

sternum may be seen forming the floor of that cavity between the mandibles (fig. 30 B, Pl. 3). For comparison with the gnathochilarium of diplopods it is also of importance to observe that the collum segment does not contribute to the formation of the intermaxillary plate; for though the intersegmental groove between the two segments is not very deep, it is sufficiently clear to define precisely the limits of the two segments. (Some further observations on the development of the intermaxillary plate are given in section 10 (iv).) The antennae have now grown narrower and longer, and at their distal ends have begun to bifurcate; their bases have approached still more closely on the anterior surface of the head.

The usual difficulty of determining the limits of the gnathal segments within the head-capsule is accentuated, in *Pauropus*, by the absence of muscle indications. There is only one intersegmental line, namely, that between the mandibular and maxillary segments, and it does not appear until about the tenth day. It is then distinguishable by the fusiform character of its cells, but can at first be seen extending up for only a little distance from between the bases of the mandibles and maxillae (fig. 29 A, Pl. 3). In pupae (figs. 32 A, 33 A, Pl. 3) it becomes more prominent, and now encircles the head, developing above into a pronounced groove. This groove remains as the occipital suture in the chitinized head-capsule, but does not extend the full distance to the bases of the appendages.

The groove demarcating the clypeus from the antennal area is present from the tenth day onwards (fig. 29 B, Pl. 3).

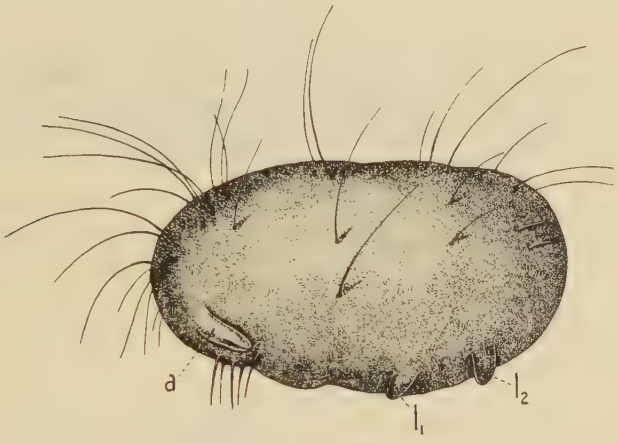
The superlinguae do not make their appearance till after the pupa has formed; they are, for example, not present in the advanced embryo shown in fig. 30 B, Pl. 3. They arise as a pair of outgrowths from the floor of the preoral cavity, being the product of the mandibular epidermis (Text-fig. 9).

A feature of the very advanced embryo is the pronounced deepening of the intersegmental grooves behind the first abdominal segment, and the formation of several transverse folds that distort the body-wall, especially on the head. It is evident that the epidermis of the advanced embryo has enlarged to a degree where it can no longer be accommodated without distortion within the old embryonic cuticle. Presumably in consequence of absorption of moisture from without, the surface distortions now gradually even out, the embryo enlarges, and so slowly forces its way from the egg to enter the 'pupoid' phase.

(iii) *The 'pupoid' phase.* The pupa (Text-fig. 3) is a smooth, white object, measuring 0·2 mm. in length. It is quite immobile. It is rather narrower in front than behind. Minute oral and anal apertures are present, the chitin being inflected through them to form a lining for the fore-gut and rectum. The pupal sheath shows the impress, from within, of the bifurcated tips of the antennae, and of the tips of the first and second, but not third, legs. It is clothed with setae, most of which lie in transverse rows and are long, slender, and curved, though there are also a few that are relatively short and stout.

The appearance of the stained embryo, seen through the transparent pupal sheath, is shown in fig. 32 A, Pl. 3. The head has assumed almost its adult

form. On the antennae the three flagella are beginning to grow out (fig. 32 B, Pl. 3). The collum and first leg-bearing segments have assumed almost their definitive form, but the hinder segments are still pressed against one another and it is due to the release from this that the subsequent elongation of the larva, after leaving the pupa, is due. The tergal wall of the second and third segments is strongly developed, but that of the following segment is reduced to a narrow strip of epidermis, being, as it were, wedged in between the third and fifth segments. The anal segment is now sharply demarcated from the



TEXT-FIG. 3. The pupa.

Lettering. *a* antenna; l_1, l_2 first and second legs.

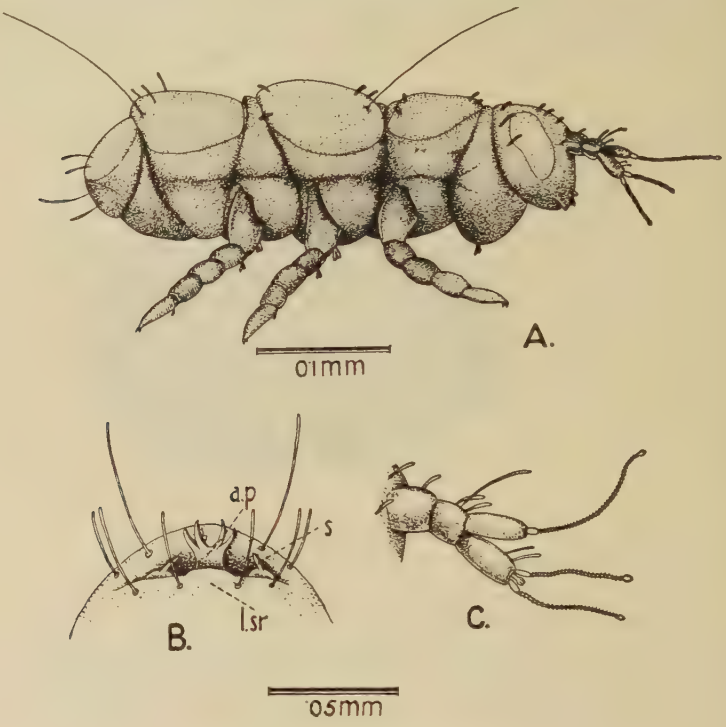
fifth. The legs have become further enlarged and bent under the ventral surface of the embryo, and are beginning to show signs of segmentation (fig. 32 C, Pl. 3). Only the five segments which form the segments of the mature limb are distinguishable, there being no indication of a 'sub-coxa' postulated by some morphologists.

In the pupa the flagella of the antennae elongate rapidly (figs. 33 A and B, Pl. 3) and the globulus is now also visible. There are no nuclei in the flagella. The latter are protoplasmic prolongations from cells at the tips of the rami, and several cells seem to co-operate to form them.

Chitinization does not begin until about the end of the second day, and thereafter the various setae and specialized 'hairs' of the larva become visible beneath the pupal sheath. Special importance attaches to the development of the second and third tergites. The former is entirely the product of the tergal wall of the third (second leg-bearing) segment, and the reduced tergal wall of the succeeding segment contributes nothing to it. Similarly the tergite of the fifth abdominal segment will become the third in the adult animal, and it, also, is the product of a single segment, the future sixth abdominal segment (which is related to the fifth as the fourth is to the third—see Text-fig. 24 A) being still only in course of formation. This solves the problem of

the supposed 'diplotergites' of *Pauropus*; they are not 'diplotergites', but derivatives of a single segment, and each tergite-bearing segment (except the first) is followed by an 'intercalated' segment with reduced tergal wall, and without a tergal shield (see further, Post-emb. Dev., sections 1, 2).

After 3 or 4 days a split appears along the dorsal surface of the pupa, and the larva is set free.



Text-fig. 4. First Instar Larva.
A. Entire larva. B. Hinder end. C. Antenna.
Lettering. *a.p* anal plate; *l.sr* lamina supranalis; *s* style.

(iv) *The First Instar Larva* (Text-fig. 4). This is a small, white, actively moving creature, measuring about 0·25 mm. in length.

The head of the larva possesses most of the characters of the adult animal. On the antenna the globulus and three flagella are present; there are, however, only two basal segments in the antenna, and the number of rings in the flagella is only about a third of the adult number (Text-fig. 4 C). The mandibles and maxillae have assumed their adult features. The pseudoculi are present.

The collum segment is wedge-shaped, and is without a tergal shield. On the second segment a shield is present. There is a similar shield on the following segment, and this bears a pair of long sensory setae (trichobothria), the specialized epidermal cells from which these arise being already evident in

10-day embryos (fig. 29 A, Pl. 3). The fourth segment is without a shield; in extended larvae its reduced tergal wall is readily seen (Text-fig. 4 A), but if the larva is contracted it is withdrawn beneath the second tergal shield, which thereby acquires the misleading appearance of a diplotergite. The third shield, that of the fifth abdominal segment, bears, like the second, a pair of long tactile setae; this shield will remain as the third shield in the next and later instars, the new segments being generated from the anal segment. There is a fourth tergal shield, namely, on the anal segment, but it is smaller and weaker than the three that precede it. This segment is exceptional in being provided with a sternite, the only sclerotized sternite in the abdomen.

In the first instar larva the first teloblastic (i.e. sixth abdominal) segment has already become defined. Like the fourth segment, it has a reduced tergal wall, and is wedged in behind the fifth segment. It may be seen in Text-fig. 4 A. For an account of its development, see below, Post-emb. Dev., section 2.

It is noteworthy that limb-buds for the new segments are not yet present, and in this respect *Pauropus* differs from the Symphyla, in which the first pair of legs that mature during anamorphosis is already present in the advanced embryo.

Up to the time when the embryo is entering the pupal phase, the anus has remained a transverse slit at the tip of the anal segment. But in the larva it is withdrawn from the surface (Text-fig. 4 B); it is overhung by the 'lamina supranalis', while below it is the 'lamina subanalis', but neither is demarcated by a groove from the rest of the anal segment. The lamina subanalis bears the peculiar 'anal plate' (Hansen, 1902); under the lamina supranalis, to the sides of the anus, are two papillae that bear the styles.

Each leg has five segments, the tarsi of the first instar larva being unsegmented in all the legs.

(v) *General Remarks.* In comparison with other myriapods and with insects, the external form of *Pauropus* develops along comparatively simple lines. The initial dorsal flexing of the germ-band is well known also in chilopods, many diplopods, and, amongst primitive insects, in Diplura and Collembola; but in all these forms it soon changes to complete ventral flexing, the latter being present, even from the beginning, in Symphyla and many Diplopoda. In *Pauropus* alone amongst myriapods does the slight dorsal flexure persist. The absence of embryonic membranes was to be expected; these are confined to the insects and have, indeed, evolved within that class.

Pupoid phases are of widespread occurrence amongst myriapods, though in no previously described case is there so distinctive a 'pupa' as in *Pauropus*. By a 'pupa' we mean a precociously liberated motionless embryo, with unsegmented or only partially segmented appendages, invested by a protecting cuticle, within which the embryo completes its development at the expense of the nutritive yolk. Amongst the diplopods a pupoid phase was observed by Newport as long ago as 1841, and has since been found in various members of the group. In *Strongylosoma* and *Polydesmus* (Metchnikoff, 1874) and in *Julus terrestris* (Heathcote, 1886) the 'pupa' is enclosed, as in *Pauropus*, within a specific

embryonic cuticle which already shows the rudiments of appendages. In *Julus morelettii* (Metchnikoff, 1874) the pupa is encased within a blastodermic cuticle, and this seems to hold also for *Archispirostreptus*, in which limb-buds do not protrude (Robinson, 1907). In *Glomeris*, on the other hand, a pupoid phase seems to be omitted (Hennings, 1904). Amongst the chilopods, *Geophilus* (Metchnikoff, 1875) and *Scolopendra* (Heymons, 1901) both show precocious rupture of the egg-membrane, thereby partially disclosing an embryo encased within an embryonic cuticle. Heymons, who has given a detailed account of these phases in *Scolopendra*, finds that the embryo now moults and emerges from the egg as a motionless 'embryonic stadium', beneath the cuticle of which development continues, the embryo living on its yolk; this in turn moults, and discloses the 'foetus', a transient non-feeding stage, out of which the feeding 'adolescent stage' later emerges. In Symphyla there is no known case of a pupa. A kind of 'pupa' is, however, very prevalent amongst Collembola, for the egg-membrane usually ruptures at an early stage of development, the enlarged embryo continuing its development under cover of the blastodermic cuticle.

In the character of its segmentation, the germ-band of *Pauropus* seems to resemble that of diplopods; and it is, in fact, identical with that figured by Silvestri (1933) for *Archispirostreptus gigas*. Its most noteworthy feature is the collum segment, quite devoid of appendages, and this character it shares with diplopods but with no other myriapod[1] or insect. The comparison with diplopods remains tentative, however, for there is a surprising lack of unanimity in the published accounts of the development of these myriapods. From the presence of a tritocerebral ganglion in the adult diplopod brain the presence of a pre-mandibular segment in the diplopod germ-band is to be expected, as in *Pauropus*; yet neither Heymons (1897) nor Lignau (1911) refer to it, and Pflugfelder (1932) states explicitly that it is not present. Owing to the vestigial form in which this segment commonly appears in insects, there may perhaps be some difficulty in identifying it in diplopods also, and greater weight should therefore probably be given to the statements of Silvestri (1903, 1933) and Robinson (1907) that such a segment is present in the embryos they examined (*Pachyjulus*, *Archispirostreptus*).

A post-maxillary collum segment, in which appendage rudiments are absent, is referred to by all recent authors on diplopod development, with one exception: Pflugfelder alone states that this segment bears a pair of second maxillae. If this is correct, it is strange that competent observers like Heymons and Silvestri can find no trace of such appendages. In *Pauropus* this segment[2] does not become part of the head; for diplopods the question is still undecided: Metchnikoff (1874) reported that two appendage-bearing segments, and two only, became associated with the head, the second pair of appendages uniting to form the lower lip (gnathochilarium). According to Heymons (1897), the

[1] A kind of 'collum segment' is found in some adult Symphyla, but it is not the equivalent of the post-maxillary collum of Diplopoda and Pauropoda.

[2] Latzel speaks of the collum segment as the basal segment of the head; it is clear, however, that the 'cephalization' of this segment is not comparable with that of the second maxillary segment of chilopods, Symphyla, and insects.

gnathochilarium has a more complex origin, in that it arises by fusion of the first maxillae with the 'hypopharynx', i.e. sternites of the mandibular and maxillary segments; Heymons is, however, quite emphatic that the 'lamellae linguales' and 'stipites gnathochilarii' do not represent separate appendages, but arise by longitudinal division of the single pair of fused maxillae. While agreeing as to the presence of only a single pair of maxillae in the gnathochilarium, Silvestri (1903) reported that not only the maxillary sternite, but also that of the collum segment, entered into its formation, the basal sclerite (hypostome) being derived from the latter. This is confirmed by Lignau (1911); but while all other authors are agreed that at least the tergal portion of the collum segment remains separate from the head and forms the 'collum' of the larva, Lignau claims that the 'collum' develops out of the first leg-bearing segment. The remaining two publications on the development of the gnathochilarium only add to the confusion. From a very fragmentary series of *Archispirostreptus* embryos Robinson (1907) reported two maxillary segments between the mandibular and collum segments; the first pair of maxillae were stated to degenerate, while out of the second was formed the gnathochilarium. Pflugfelder (1932), on the other hand, states that the collum segment is furnished with appendages; the gnathochilarium, according to this author, arises by fusion of the first and second maxillae, the sternite of the collum segment apparently forming the 'hypostome'.

There are no adequate reasons for referring these six conflicting accounts to diversity in the forms examined. Most authors are agreed that the legless post-maxillary segment of the embryo becomes, at least in part, the collum of the adult animal; and in this respect the diplopods will then resemble *Pauropus*, but no other myriapod. There remains, however, the more debatable question of the relationship between the intermaxillary plate and maxillae of *Pauropus* and the gnathochilarium of diplopods. Does the gnathochilarium contain, in addition to the first maxillae, the equivalent of a labium? The most recent work, that of Pflugfelder, asserts that it does; on the other hand, Metchnikoff, Heymons, Silvestri, and Lignau agree that there are no second maxillae. Even if we accept Silvestri's statement that the sternite of the post-maxillary (collum) segment is incorporated into the gnathochilarium as its 'hypostome', this is by no means the equivalent of the cephalization of a labial segment and of the enlargement of the pre-oral cavity by a labium. Silvestri's work, in particular, is attested by some very clear drawings, and these are impossible to reconcile with Pflugfelder's recent work. As far as we can evaluate the conflicting accounts, it would seem that the diplopod gnathochilarium is the equivalent of the intermaxillary plate and maxillae of *Pauropus*, complicated by the incorporation of a post-maxillary sternite; and it might be regarded as an organ elaborated from some simple forerunner, of the kind found in *Pauropus*; but if Pflugfelder's work is confirmed, then the mouth-appendages of diplopods must be interpreted as a highly specialized modification of the system of appendages that form the mouth-parts in other myriapods and in insects.

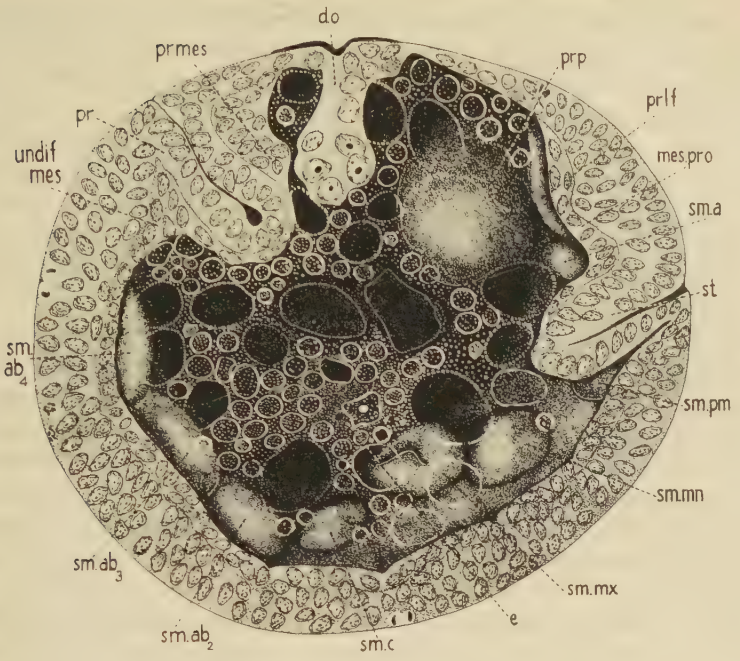
The absence of a second maxillary segment in the head of *Pauropus* is of importance for the assessing of its affinities. It is clear that cephalization has not proceeded as far as in chilopods, Symphyla, and insects. In the embryology of Symphyla there is evidence of the relatively recent incorporation of this segment into the head, for it appears first as part of the abdomen rather than of the head (Tiegs, 1940), and even in insects the abdominal rather than cephalic character of its mesoderm has long been recognized (Wiesmann, 1926).

7. *Differentiation of the Mesoderm, and Formation of Somites*

By the time the germ-band has become defined, the formation of mesoderm is at an end. As already described in section 4, the mesoderm extends from just behind the stomodaeum almost to the posterior end of the germ-band. For some distance behind the stomodaeum it is heaped up several cells deep (fig. 54, Pl. 5), and even extends forward a short distance round the stomodaeum; elsewhere along the germ-band it forms a rather irregular layer, with a tendency for its cells to accumulate along the lateral margins of the germ-band in the position of the future somites.

With the pre-oral elongation of the head-lobes that takes place during the seventh day, mesodermal cells begin to spread forward from behind the stomodaeum, along the floor of the head, almost to its anterior tip. This pre-oral mesoderm thereby comes to form a continuous sheet of cells on the floor of the head (fig. 58 A, Pl. 5), but it does not spread over its lateral walls, where the process of thickening, associated with the formation of the protocerebral ganglia, is already beginning. Some cells also spread on to the stomodaeum itself (fig. 59, Pl. 5), and are the source of the mesodermal sheath of the fore-gut and eventually also of the mid-gut (see section 9). The post-oral accumulation of mesoderm is thereby much diminished. By this time the proctodaeum also has begun to form, and, like the stomodaeal ingrowth, is surrounded by a ring of mesoderm. Fig. 58 A and B, Pl. 5, will serve to show the disposition of the mesoderm in embryos at this stage of development; the drawings represent two sections from a series cut 'horizontally' through the embryo, the germ-band being therefore twice transected in a single section. Fig. 58 A shows the germ-band cut (above) through the ingrowing proctodaeum, and (below) through the head-lobe some distance in front of the stomodaeum; the median pre-oral sheet of mesoderm, and the mesoderm surrounding the base of the proctodaeum are seen in this section. In fig. 58 B the germ-band is seen transected (above) at about the level of the future third abdominal segment, and (below) a little distance behind the stomodaeum; note in this section the marked diminution of the post-oral mesoderm, but the relatively abundant mesoderm in the abdomen.

Throughout the seventh day mitoses are encountered in great numbers in the mesoderm. The tendency of its cells to aggregate into two lateral bands becomes more marked, and within these a segmentation into successive somites is becoming progressively clearer. Before the end of the seventh day most of the somites have formed (Text-fig. 5).



TEXT-FIG. 5. Seven-day embryo, bisected.

The embryo is at the stage in which the stomodaeal and proctodaeal ingrowths are approaching the central yolk-laden endoderm, the latter being distinguished from the rest of the 'yolk-cells' by its large nuclei and richer cytoplasmic content. The pre-antennary somites have not yet developed out of the pre-oral mesoderm, but the antennary, pre-mandibular, mandibular, maxillary, and collum somites are fully formed, and the next three abdominal somites are in course of development. Behind this the mesoderm has not yet begun to show sign of somite formation. The 'dorsal organ' is conspicuous, and its secretion has begun to form. The large posterior lobe of the protocerebral ganglion is shown, and in front of it the ectodermal thickening out of which the frontal and lateral lobes will arise. In the ventral ectoderm nerve-cord formation has hardly begun; in places the 'median mesoderm' is shown, but in longitudinally cut embryos this is usually difficult to distinguish from the underlying ectoderm.

Lettering. d.o 'dorsal organ'; *e* endoderm; *mes.pr.o* pre-oral mesoderm; *pr* proctodaeum; *pr.l.f* developing lateral and frontal lobes of protocerebrum; *pr.mes* proctodaeal mesoderm; *pr.p* posterior lobe of protocerebrum; *sm.a*, *sm.ab*$_{2, 3, 4}$, *sm.c*, *sm.mn*, *sm.mx*, *sm.pm* somites of antennary, second to fourth abdominal, collum, mandibular, maxillary, and pre-mandibular segments respectively; *st* stomodaeum; *undif.mes* mesoderm not yet differentiated into somites.

(This drawing, as well as Text-figs. 7, 8, and 9, are reconstructions from embryos cut in sagittal series. Cell-nuclei, drawn with camera lucida from the median section of the series, are used to indicate the cut surface of the bisected embryo; structures that are not cut by the median section are indicated without nuclei.)

Complete withdrawal of mesoderm from the mid-line does not take place. There survives here a narrow, and at first discontinuous, band of cells, which I shall speak of as 'median mesoderm'. Unlike the lateral bands of somites, it remains unsegmented. In some places its cells adhere closely to the adjacent ectoderm, filling out irregularities along its surface, and they may, for that

reason, often be difficult to distinguish from ectodermal cells; particularly are they apt to fill in a median groove between the two lateral thickenings of ectoderm, out of which the nerve-ganglia will form (figs. 63, 64, 65, 66, Pl. 5; fig. 67 B, Pl. 6; figs. 74, 75, 76, Pl. 6).

Within most of the segments (mandibular to fifth abdominal) there remains a small amount of mesoderm which at no time forms part of the somites. It moves into a position dorso-lateral to the somites, where it lies against the epidermis, and provides the material from which the dorso-lateral muscles develop in the late embryo. It is shown in fig. 64, Pl. 5, and is referred to more fully in section 15 (*b*) (ii).

In the procephalon there develop three pairs of somites, namely, pre-antennary, antennary, and pre-mandibular, but of these the pre-antennary are very vestigial. The mandibular, maxillary, and first four abdominal segments contain each a single pair of somites, and these are clearly distinguishable by the end of the seventh day (fig. 79, Pl. 7; Text-fig. 5). The fifth abdominal and anal segments also develop each a pair of somites, but their formation is delayed at least a day after that of the others. The presence of a distinct somite in the anal segment is evidence that the latter is a true segment and not a telson.

The fully formed somite usually presents a distinction between an outer thick somatic wall and a thinner visceral wall of flattened cells adjacent to the yolk (figs. 60, 64, Pl. 5; figs. 80, 81, Pl. 7). But even at best the visceral wall is but poorly developed, and somites are sometimes encountered where it cannot with certainty be seen. At the intersegments successive somites of the gnathal and first four abdominal segments abut on one another, and are not separated by gaps. At the height of their development many of the somites show minute cavities (fig. 81, Pl. 7), but there is no indication of those spacious coelomic vesicles that we find in other myriapods. Nor do the minute coelomic cavities of successive somites communicate at the intersegments.

A detailed account of the development and transformation of the individual somites is given in section 8.

The 'median mesoderm' gives origin to the genital tube and to certain cells, apparently neuroglial in nature, associated with the nerve-cord. For an account of these, see sections 11 and 13.

8. *Transformation of the Somites*

(i) *The Pre-antennary Somites.* These are the least developed of the somites of the procephalon, and are also the last to form within it. For some time after the succeeding two pairs of somites have already formed, the pre-oral mesoderm still lies clumped into a small median mass of cells in front of the stomodaeum (Text-fig. 5; fig. 68, Pl. 6). But during the eighth day we find, in its place, a pair of closely apposed rounded cell masses, in which there is a just recognizable distinction between visceral and somatic wall, though a coelomic cavity never develops (fig. 67 A, Pl. 6; fig. 93, Pl. 8).

During the ninth day these diminutive somites disrupt into a single clump of cells, which fills the cavity of the clypeus (fig. 103, Pl. 9). In later embryos we find these cells in process of elongation and conversion into the buccal dilator muscles (Text-fig. 7). The retractor of the clypeus (see section 15) probably also arises from this source.

These somites are evidently the equivalent of the pre-antennary somites of *Scolopendra* (Heymons, 1901), *Platyrrhacus* (Pflugfelder, 1932), and *Hanseniella* (Tiegs, 1940), and correspond also to the pre-antennary (labral) somites described for various insects—*Carausius* (Wiesmann, 1926), *Rhodnius* (Mellanby, 1936), *Locusta* (Roonwal, 1937), but in accordance with the great reduction which the somites have undergone in *Pauropus* have here become reduced to vestiges, and are not associated with any recognizable appendages. In Symphyla, where the mesoderm is very generalized, they play a role in the development of the dorsal blood-vessel, for not only do the buccal dilator muscles arise from them, as in *Pauropus*, but they also give origin to the 'funnel' of the aorta. In insects, on the other hand, the cephalic aorta arises wholly from the antennary mesoderm; in the one instance in which the further development of the pre-antennary (labral) somites has been followed, they have been found to develop into labral musculature (*Locusta*, Roonwal, 1937). In *Scolopendra* also they do not seem to aid in the formation of the dorsal vessel.

(ii) *The Antennary Somites.* These are the first somites to develop in the procephalon, and are also its largest. In embryos in which the rudiments of the antennae are just becoming perceptible in external view, the somites are seen in section, lying flattened out against the thickened epidermis of the developing appendages. They already show a clear distinction between a thin visceral and a thick somatic wall (fig. 59, Pl. 5).

With the ensuing migration of the antennae into a pre-oral position, these appendages begin to elongate and develop a cavity. Into the hollows of the antennae the somites fit. They no longer lie flattened against the epidermis, but have now assumed the form of mature somites (fig. 67 D, Pl. 6), and a little later even display each a very minute coelomic cavity (fig. 60, Pl. 5).

Transformation of the somites proceeds along very simple lines. During the ninth day they disrupt each into a loose clump of cells, which multiply and completely obliterate the cavity of the elongating appendage (fig. 110, Pl. 9); out of these will form the muscles within the antennae. Other cells spread from the bases of the antennae backwards along the roof of the head, to the side of the protocerebral ganglia, and are the source of the tergal muscles of the antennae, and also, probably, of the large dorso-lateral muscles of the head (see section 15).

The antennary mesoderm does not, as in Symphyla, contribute any cells to the formation of the stomodaeal musculature, and in accordance with the total suppression of blood-vessels it does not contain any vasoblasts. In this respect its development has evidently become much simplified, for its contribution to the formation of the vascular system in other myriapods and in

insects is very considerable. In *Scolopendra*, according to Heymons (1901), part of the cephalic aorta arises from it; in Symphyla it gives origin to the greater part of this vessel, including the antennary arteries, while in insects even the entire cephalic aorta develops from this source.

(iii) *The Pre-mandibular Somites.* In embryos in which the stomodaeum has begun to invaginate, the pre-mandibular mesoderm becomes recognizable as a pair of flattened cell-masses that lie against the thickened ectoderm a little post-orally, and in line with the row of other developing somites (fig. 61, Pl. 5). There does not seem to be any intervening 'median mesoderm'.

With the displacement of the pre-mandibular ectoderm that attends the development of the pre-oral cavity (cf. section 6 (ii) (*b*)), these masses of pre-mandibular mesoderm become drawn closer together, and now lie as a pair of rounded somites in a depression of the ectoderm immediately postero-lateral to the pre-oral cavity (Text-fig. 5; fig. 79, Pl. 7). As the pre-oral cavity becomes better defined, they come to occupy a position completely lateral to it (fig. 67 D, Pl. 6).

Meanwhile the somites have begun to elongate, and within each a small cavity appears (fig. 62, Pl. 5). During the eighth day each somite undergoes considerable elongation, growing backward into the cavity of the head to the side of the pre-oral cavity, on to the floor of the mandibular segment. The full extent of the elongating somite is to be seen in the succession of sections shown in fig. 67 C–F, Pl. 6. It is also shown in single section in fig. 71, Pl. 6, from an embryo of about the same age, in which the section was accidentally orientated to traverse most of the length of the somite; to the right the large cell-mass is the inferior wall of the pre-oral cavity, to the left is the developing mandibular ganglion and its 'ventral organ'. The prospective glandular nature of the somite is now plainly recognizable. Unlike the other somites of the procephalon, it does not disrupt, but continues to enlarge and becomes the pre-mandibular salivary gland.

The later development of the pre-mandibular gland is described in section 10 (ii).

In the embryo of *Scolopendra* (Heymons, 1901), and *Hanseniella* (Tiegs, 1940) comparatively well-developed pre-mandibular somites, furnished with coelomic cavities, are known, but they do not seem to have been recorded in any diplopod. Hoffman (1911) observed them in the embryo of the Collembolan *Tomocerus*, and even in primitive winged insects there are recognizable vestiges of them—*Xiphidium* (Wheeler, 1893), *Forficula* (Heymons, 1895), *Carausius* (Wiesmann, 1926), *Locusta* (Roonwal, 1937). But in the higher order of insects they are reduced to mere cell-aggregations. Their actual conversion into recognizable segmental organs is at present known only for *Pauropus* and *Hanseniella*, though Wheeler and Heymons had recognized the vestige of such an organ in the developing sub-oesophageal bodies and 'lymphoid tissue' of *Xiphidium* and *Scolopendra* respectively.

(iv) *The Mandibular Somites.* Like the developing somites of the antennary segment, the mandibular somites lie at first flattened out against the thickened

epidermis from which the associated appendages are beginning to form. Between them is some 'median mesoderm'. As the mandibles develop the somites take definite shape, and now show a well-formed visceral and somatic wall (fig. 79, Pl. 7). They fill the diminutive hollows of the growing mandibles, and are rather larger than most of the other somites. Within each a minute coelomic cavity appears (figs. 67 F, Pl. 6; figs. 80, 81, Pl. 7). In this condition they remain longer than the immediately adjacent maxillary and pre-mandibular somites, for these are to be seen in process of conversion into their respective glands, while the mandibular somites are still intact.

Transformation of the somites begins during the eighth day, and from them there arises nothing but the mandibular musculature. The manner of development of this musculature is bound up with the formation of the hypopharyngeal apophyses and with the peculiar character of the mandibles themselves, which in the adult *Pauropus* have each a long blade-like apodeme invaginated deeply into the head-capsule (cf. section 6 (ii) (*b*)). The formation of the apodemes begins in the 9-day embryo, by the ingrowth of the ectoderm around the lateral margins of the appendages. The form of this ingrowth will readily be visualized by reference to fig. 70, Pl. 6, and fig. 110, Pl. 9; fig. 70 has been drawn from a section that passes transversely through the floor of the maxillary segment, while fig. 110 is from a section cut 'horizontally' through the floor of the segment, i.e. from a frontal section of the head.

This ingrowth of the mandibular apodeme is already seen in the earlier embryo shown in fig. 69, Pl. 6; the outlines of the somite are still recognizable in this embryo, even though the coelomic cavity has disappeared. But in the more advanced embryos shown in fig. 70, Pl. 6, and fig. 110, Pl. 9, it has disrupted into an unorganized clump of cells, which, increasing in quantity, are in process of being drawn into the cavity of the head with the ingrowing apodeme.

The further development of the musculature of the mandible is described in section 15 (*b*) (i).

In all those myriapods that have been adequately examined on this point, a well-developed mandibular coelomic sac has been found—*Scolopendra* (Heymons, 1901), *Julus* (Heathcote, 1888), *Platyrrhacus* (Pflugfelder, 1932), *Hanseniella* (Tiegs, 1940); even in Orthoptera a large coelomic sac is present in this segment (Wheeler, 1893; Heymons, 1895; Wiesmann, 1926; Roonwal, 1937), though in higher insects it tends to disappear. In giving origin to the mandibular musculature, the somite in *Pauropus* conforms to the general scheme for other myriapods and insects; but in other respects it has evidently undergone much simplification, for in addition to the absence of any vasoblasts in its walls, it does not contribute any splanchnic mesoderm to the wall of the mid-gut or fore-gut.

(v) *The Maxillary Somites.* By the seventh day the first indication of the maxillary somites has become evident, by the accumulation of the mesoderm of the segment into two masses, which are separated by a narrow band of unsegmented median mesoderm (fig. 63, Pl. 5). During the course of the day

the segment grows in width; its ganglionic thickenings begin to form, and thereafter the developing somites move into a more lateral position in the segment, into the place where the maxillae will soon appear (fig. 64, Pl. 5). They lie, indeed, considerably more to the side than do the somites that precede them (Text-fig. 5; fig. 67 F, Pl. 7). Within each a thick somatic wall and a thin visceral wall of flattened cells are distinguishable (fig. 64, Pl. 5; fig. 79, Pl. 7). Thereafter, as the maxillae begin to form, they become more rounded, and develop each a small coelomic cavity, and therewith attain the height of their development.

Their transformation sets in unusually quickly. From the hinder end of each somite a tubular ingrowth, with just perceptible lumen, begins to grow in the direction of the yolk. These elongating somites are a characteristic feature of all embryos during the eighth day, and serve as a ready means for identifying the maxillary segment in sections (fig. 67 F, Pl. 7; fig. 80, Pl. 7).

Before long the somite becomes converted into a plainly recognizable gland rudiment. It is the maxillary (salivary) gland. It is now a narrow tube, with just perceptible lumen, and is completely doubled on itself (Text-fig. 7). At its lower end, occupying the hollow of the developing maxilla, is the remains of the somite (fig. 88, Pl. 7); this has itself become rather enlarged, mitoses are not infrequent among its cells, but the characteristic cell-disposition of the original somite is much obscured.

In parasagittal sections the separation of this clump of cells from the base of the tubular gland-rudiment is becoming apparent (fig. 81, Pl. 7). The lower end of the gland is now seen to be situated between the maxilla and the mandible, but has not yet acquired an opening to the exterior. The clump of cells that is separating from it comprises myoblasts for the formation of the maxillary musculature, and these occupy the cavity of the maxilla.

In appropriately cut sections of more advanced embryos we find the lateral margin of the maxilla growing into the cavity of the head, to form an apodeme, similar to that of the mandible, but much smaller (figs. 110, 115 B, Pl. 9). The mass of myoblasts has now separated away completely from the gland rudiment; some of them remain within the maxilla, but others become drawn farther into the cavity of the head by the ingrowing apodeme.

The further development of the salivary gland and of the muscles of the maxilla is described in sections 10 (iii) and 15 (*b*) (i).

In all myriapods and primitive insects that have been properly investigated, a well-formed maxillary coelomic sac has been found. In *Julus* (Heathcote, 1888) and *Hanseniella* (Tiegs, 1940), the tubular salivary gland has been found to arise from it, as in *Pauropus*. The recent work of Fahlander (1938) suggests the probable occurrence of mesodermal glands also in *Scutigera* and *Lithobius*, but embryological observations on these forms are still lacking; on the other hand, in *Scolopendra* Heymons' observations (1901) reveal the presence only of ectodermal maxillary glands, as in insects. In other respects, however, this somite in *Pauropus* seems to have undergone simplification, for it does not supply any splanchnic mesoderm to the alimentary canal.

(vi) *The Somites of the Collum Segment.* The first sign of these somites is encountered on the sixth day, when the mesoderm of the narrow collum segment becomes heaped up into two masses, with a little intervening median mesoderm (fig. 65, Pl. 5; drawn from the same embryo as fig. 63, Pl. 5).

With the development of the collum ganglion, the segment widens, and the somites move farther to the side (fig. 66, Pl. 5). Here they lie in a depression of the ectoderm, but of appendages there is no trace whatever. The mature somites are rather smaller than those of the adjacent segments, and at no time show any coelomic cavity (fig. 66, Pl. 5; figs. 79, 81, Pl. 7).

In this condition they survive well into the eighth day. Thereafter they disrupt into an unorganized clump of myoblasts which lie to the side of the ganglion (fig. 89, Pl. 7), and provide the material from which the muscles of the collum segment will form.

Although the collum segment is the equivalent, in position, of the labial segment of insects, the transformation of its somite has proceeded along remarkably simple lines; for it does not contribute any splanchnic mesoderm to the mid-gut wall, nor is there any indication of a process that might be regarded as the vestige of the formation of a segmental organ. In Collembola, on the other hand, according to Philiptschenko's work on *Isotoma* (1912), a labial salivary gland arises from it.

(vii) *The Somites of the Second, Third, and Fourth Abdominal (Three Leg-bearing) Segments.* In the region of the leg-bearing segments the germ-band is, during the seventh day, much wider than in the collum segment. The quantity of mesoderm is here also greater; it is, however, not so heaped up, but lies flattened out against the adjacent ectoderm. This is well seen in fig. 74, Pl. 7, which represents a section through the second abdominal segment, and from the same embryo as shown in figs. 63, 65, Pl. 5. Between the lateral accumulation of mesoderm is a little median mesoderm.

In rather older embryos we find the paired masses of mesoderm in process of conversion into somites (fig. 75, Pl. 7). They still lie flattened out against the ectoderm, but there is already evident an alignment of the cells which foreshadows that of the future somite.

In the section shown in fig. 67 B, Pl. 6, these flattened cell-masses are becoming rounded off, and have now assumed the form of mature somites, both visceral and somatic walls being distinguishable, though coelomic cavities have not yet appeared.

By this time recognizable ganglion rudiments are present in the abdominal segments. With the enlargement of these, the somites, as in the more anterior segments, become forced more to the sides, where they are now lodged in depressions of the ectoderm. From this ectoderm the legs are now in process of forming (fig. 76, Pl. 7).

Lodged thus in the hollows of the appendage rudiments, the abdominal somites often develop each a small coelomic cavity. In fig. 76, Pl. 7 (right side), is shown the coelomic cavity of the somite of the fourth abdominal segment of a late 8-day embryo, and in fig. 81, Pl. 7, all the somites of the

abdominal segments display such a cavity. Yet frequently the somites seem to remain permanently devoid of one, and at times even the visceral wall cannot be distinguished (fig. 76, Pl. 7, left side), its nuclei being withdrawn to the sides of the somite.

In parasagittal sections small bridges of intersegmental mesoderm may be seen between the somites (fig. 81, Pl. 7); but the minute cavities of successive somites are not continuous through these bridges.

As the limb-buds grow, the lower ends of the somites become drawn out with the elongating appendages, and therewith the regular cell-alignment of the somite wall begins to disappear (fig. 77, Pl. 7). This is the first stage in the disruption of the somites.

In rather more advanced embryos the somites break down wholly into masses of cells, which fill the hollows of the appendages, and extend from the bases of the appendages medially on to the developing ganglia (fig. 78, Pl. 7), while between successive somites the quantity of mesoderm has also begun to increase. From all this large mass of cells there arise solely myoblasts for the formation of the musculature of the abdominal segments. The development of this is described in section 15.

The most surprising feature of these somites is that they do not supply any splanchnic mesoderm to the wall of the mid-gut. This seems to be quite unique among myriapods and insects.

(viii) *The Somites of the Fifth Abdominal and Anal Segments: the Teloblastic Mesoderm.* The fifth abdominal and anal somites may conveniently be considered together. They differ from all the preceding somites in their relatively late appearance, for they do not develop until the ninth day, when most of the other somites are already in process of disruption.

Up to the eighth day, when all the more anterior somites have already appeared, the mesoderm of these segments is still an undifferentiated mass of cells that extends back from the fourth abdominal segment to the hind end of the germ-band, encircling the proctodaeum. It may be seen in Text-figs. 5 and 7, and in fig. 83, Pl. 7, the latter being a section cut transversely through the fifth segment, a little in front of the proctodaeal opening.

A similar section through a 9-day embryo (fig. 84, Pl. 7) shows that the mesoderm has spread out across the floor of the segment. Its most striking feature is now a single large primordial germ-cell, lodged at its middle, with an investment of sheath cells. This germ-cell has not hitherto been distinguishable, and it is therefore impossible to determine whether it is itself an immigrant into the mesoderm or whether it has arisen directly from the latter. At this period, also, there is to be seen a tendency for the mesoderm to develop a pair of lateral thickenings, and a section through an embryo only a little more advanced shows that these thickenings are the developing fifth abdominal somites (fig. 85, Pl. 7). In longitudinally cut embryos these somites are seen to lie immediately to the rear of the fourth abdominal somites; they are, however, considerably smaller than these, and do not display a coelomic cavity (fig. 82, Pl. 7).

After the formation of the fifth pair of abdominal somites, yet another pair develops to the rear of these, a little postero-lateral to the proctodaeum. These are even smaller than the fifth somites, and do not contain a coelomic cavity. They are the somites of the terminal (anal) segment (fig. 82, Pl. 7).

In the advanced embryo the fifth somites break down each into an irregular clump of cells (fig. 86, Pl. 7), out of which the musculature of the segment later develops (see Post-emb. Dev., sections 2, 3). The anal somites also disrupt, and may be seen in late embryos as two clumps of mesoderm cells ventro-lateral to the rectum (Text-fig. 8). It is probable that these small clumps of anal mesoderm give origin to the 'occlusor ani' muscles, two small muscles lying to the sides of the anal opening and by their contraction pressing the lamina subanalis and lamina supranalis together (see Text-fig. 26).

After formation of the somites an unusually large amount of mesoderm remains heaped up around the single primordial germ-cell (fig. 85, Pl. 7). Only a small portion of this mesoderm is actually used in the subsequent development of the genital rudiment; the greater part of it separates away and moves into a more lateral position against the body-wall, where it is recognizable in the pupa as the material from which the mesoderm of the teloblastic segments will be generated in the larva. I shall refer to it as the 'teloblastic mesoderm' (fig. 87, Pl. 7); see further, Post-emb. Dev., section 3.

(ix) *General Remarks on the Somites.* In *Pauropus* the somites are remarkable among myriapods for their extreme simplicity. Bearing in mind the strongly developed coelomic sacs of other myriapods, of primitive insects, and of *Peripatus*, this simplicity must almost certainly be attributed to reduction, and is evidently correlated with the absence of a cardiac Anlage in the embryo. The absence of vestigial coelomoducts is also noteworthy, for these are present in *Hanseniella* and in many primitive insects.

The failure of the somites to contribute any splanchnic mesoderm to the intestinal wall is also remarkable, and seems, indeed, to be unique among myriapods; but this, in turn, is doubtless a simplification, and is apparently associated with the failure of the somites to spread over the mid-gut wall, where the process of heart-formation along its dorsal surface is in abeyance. That the dorsal-longitudinal muscles arise from cells that were never part of the somites (see section 7) probably has a similar explanation. The impression is, indeed, given that the whole history of the somites has been most profoundly affected in *Pauropus* by the dwarfing of the body, and its secondary effects on the vascular system.

Despite the vestigial character of the somites, it is noteworthy that they do not undergo obliteration in any segment; there is nothing comparable with the partial or even complete loss of somites that we find in the embryos of the more specialized orders of insects.

The failure of the somites to contribute to the formation of fat-body is not unexpected, for this is encountered also in Symphyla and, judging by

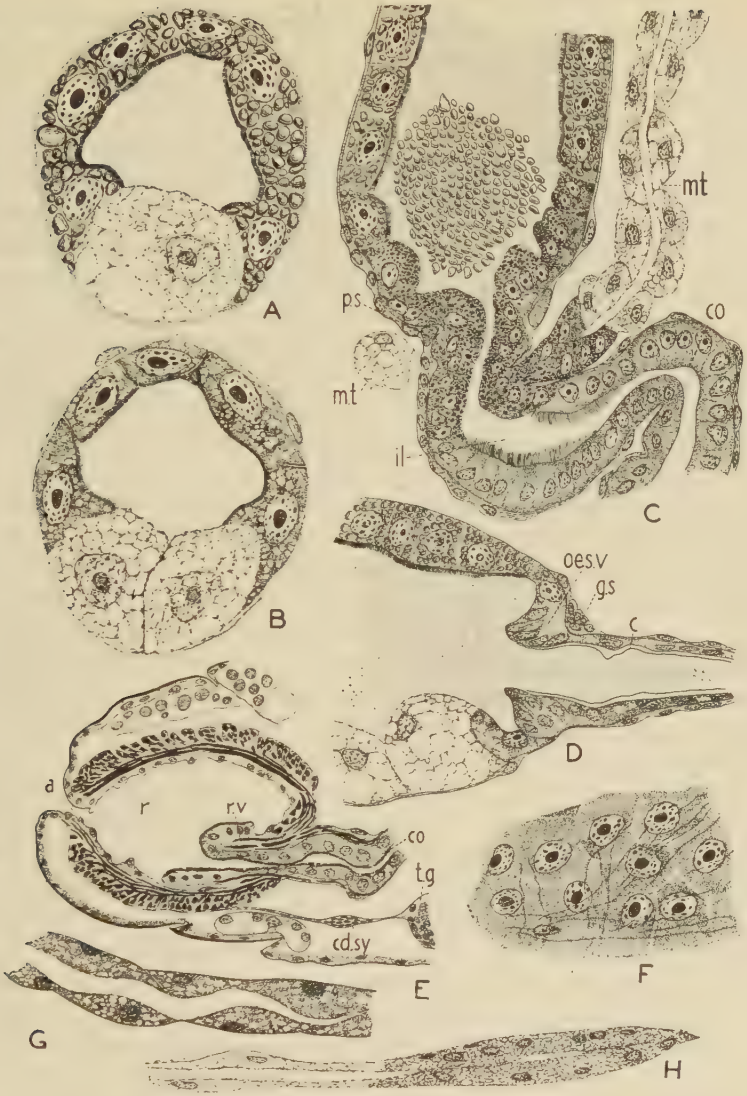
Heathcote's fragmentary work (1888), in the Diplopoda (*Julus*). Only in chilopods and insects does the fat-body arise from the somites.

9. *The Alimentary Canal and Malpighian Tubes*

(*a*) *Adult Anatomy*. The fore-gut is a simple narrow tube, which joins the mid-gut in the third abdominal segment. Its hind end is sometimes considerably expanded (Text-fig. 16 B), and an oesophageal valve is present, though seldom very pronounced (Text-fig. 6 D). The fore-gut is composed of a simple flat epithelium, lined throughout by chitin. Its musculature is weak, there being a layer of circular fibres, and external to these a just perceptible sheath of longitudinal fibres. The oesophageal dilator muscles are referred to in section 15.

The mid-gut is a simple sac, which extends from the third abdominal segment almost into the eleventh (pre-anal) segment. Its sides and roof are composed of relatively very large cells, with rather deeply staining cytoplasm, and very large nuclei, and lined internally with a 'honey-comb' border. The cytoplasm of these cells is heavily charged with coarse refringent concretions (Text-fig. 6 A), and these may, at times, be present in such quantity as almost wholly to obscure the cytoplasm; sometimes, however, the concretions are completely absent, having evidently been shed in mass, and in such cases the deeply staining cytoplasm of the cells is especially apparent (Text-fig. 6 B). These concretions are not artifacts, as some writers have suspected, for they are readily seen in animals freshly teased in saline. As Schmidt (1895) correctly observed, individual concretions are continually being discharged into the lumen of the mid-gut, and animals are sometimes met with in which they form a large massive bolus, eventually to be extruded through the anus (Text-fig. 6 C). There can be little doubt that the concretions are a waste product. The mid-gut epithelium is therefore the principal excretory organ in *Pauropus*, for the Malpighian tubes do not seem capable of eliminating waste substances, and only seldom is there any evidence for the storage of such material in visible form in the fat-body. The concretions are evidently a product of the animal's own metabolism, for they begin to accumulate even in the embryo.

In reflected light they are white. They do not show the usual radial striation of uric acid crystals. I have examined their solubility by sealing whole intestines, extracted from freshly killed animals, in depression slides ringed with vaseline (the intestine can, with practice, be drawn out in one piece by pulling upon the oesophagus). They are insoluble in water, alcohol, 4 per cent. caustic soda, and 10 per cent. acetic acid. In 10 per cent. ammonia they disappear within 2–3 hours, and in 10 per cent. hydrochloric acid within a few minutes. It is clear that they cannot be pure uric acid, though they may well be some urate. From a batch of 15 extracted intestines, from which the hind-gut with Malpighian tubes were removed, I have obtained a positive, though weak, Benedict test for uric acid. Traces of adhering fat-body could hardly be the source of the urate, for an equal number of whole animals gave



TEXT-FIG. 6. Histology of Adult Alimentary Canal.

All figures drawn to scale, except E, which is half that of the others. A. Transverse section through mid-gut, showing concretions. B. Similar section, from which the concretions have been discharged. C. Section along junction of mid-gut and hind-gut, from a 'horizontally' cut animal; within the mid-gut is a large bolus of discharged concretions; both Malpighian tubes are present in the section, one cut transversely, the other longitudinally. D. Sagittal section along junction of oesophagus and mid-gut. E. Sagittal section along hind end of abdomen, to show structure of rectum. F. Fragment of a section grazing the mid-gut wall, to show the muscle-fibrils. G. Portion of unusually well-developed Malpighian tube. H. Portion of Malpighian tube to show terminal 'glandular' portion.

Lettering. *a* anus; *c* chitin sheath; *cd.sy* caudal sympathetic ganglion; *co* colon; *g.s* stomachic ganglion; *il* ileum; *m.t* Malpighian tube; *oes.v* oesophageal valve; *p.s* pyloric sphincter; *r* rectum; *r.v* rectal valve; *t.g* terminal ganglion of ventral nerve-cord.

a positive test of no greater intensity. In one animal I have seen the extrusion of many concretion-laden cells from the intestinal epithelium into the lumen, and such cells are possibly the source of the animal remains occasionally found within the mid-gut.

The floor of the mid-gut is formed of cells of quite another kind. They are much larger even than the foregoing cells, but their cytoplasm is highly vacuolated and feebly staining; they are devoid of a striated border, and never display any concretions (Text-fig. 6 A, B, D). This structural differentiation of the mid-gut wall points to a separation into absorbtive and digestive zones, the latter having in addition an excretory function.

At the hindermost tip of the mid-gut the character of the epithelium changes, and its cells now resemble those of the most anterior part of the hind-gut (Text-fig. 6 C). They are devoid of concretions and of a striated border, the cell-nuclei are not exceptionally large, and the cytoplasm of the cells is heavily charged with deeply staining granules.

The mid-gut musculature is weakly developed, being, indeed, difficult to see. In sharply stained sections that graze along the outer surface of the mid-gut wall, a system of extremely fine striated fibrils can be seen; they run in all directions, and seem to be themselves devoid of nuclei (Text-fig. 6 F). As far as I have been able to observe, they are fibrillar differentiations, within a nucleated sheath of cytoplasm which encloses the entire mid-gut (Text-fig. 6 A, B). Despite their fineness, these fibrils may impart a strong churning movement to the intestinal wall, this being readily visible, on occasion, through the transparent body-wall of the animal.

A sphincter separates the mid-gut from the hind-gut. The most anterior part of the latter shows heavily granulated cells, and this points to some digestive function. Beyond this pyloric region is a short 'ileum', with strongly developed 'brush border'. Then follows a short weakly muscular 'colon', whose end is invaginated, as a 'rectal valve' into the rectum (Text-fig. 6 E). The latter is spacious, and, as usual, strongly muscular. The chitin of the hind-gut does not extend beyond the rectum.

There is a single pair of Malpighian tubes lying along the ventro-lateral surface of the mid-gut (Text-fig. 26), and extending forward almost into the third abdominal segment. They are not separately connected to the hind-gut, but are attached to a short outgrowth from the latter (Text-fig. 6 C). A lumen is always present, and it traverses the whole length of each tube; it does not, however, open into the hind-gut, but ends blindly. The microscopic structure of the Malpighian tubes presents much variation; in exceptional cases their structure may almost recall that of a normal Malpighian tube, though I have never met any that show a striated border (Text-fig. 6 G). But usually their constituent cells are heavily vacuolated, pale, and scarcely stainable, and give obvious evidence of atrophy (Text-fig. 6 C, H). The terminal part of each tube, for the length of nearly a segment, shows a different structure: its cells are smaller, the nuclei being much more closely approximated, and it presents the appearance of glandular tissue, though degenerated, and quite unlike the

rest of the Malpighian tube (Text-fig. 6H); see further, Post-emb. Dev., section 4.

(*b*) *Development*

(i) *The Fore-gut.* Stomodaeum formation is sometimes encountered while the germ-band is still in process of differentiation out of the blastoderm, though in most embryos it is delayed till after the germ-band has formed. By the sixth day it is always present.

It arises as a small conical thickening of the ectoderm (fig. 54, Pl. 5), only a small distance from the anterior tip of the germ-band. By the parting of its cells a lumen now appears within it, its elongate slit-like orifice being readily seen in entire embryos (figs. 23, 24B, Pl. 2). The inferior wall of the stomodaeum is markedly thinner than the superior wall (Text-fig. 5).

As the stomodaeum elongates, it acquires a loose sheath of investing mesoderm cells, which become the source, not only of the fore-gut musculature, but of that of the mid-gut as well. These cells migrate on to it from the immediately surrounding mesoderm, at the time when the somites are developing (fig. 59, Pl. 5; Text-fig. 5); the subsequent disruption of the somites that takes place during the ninth day does not, as far as I have been able to observe, yield any additional cells to the wall of the fore-gut.

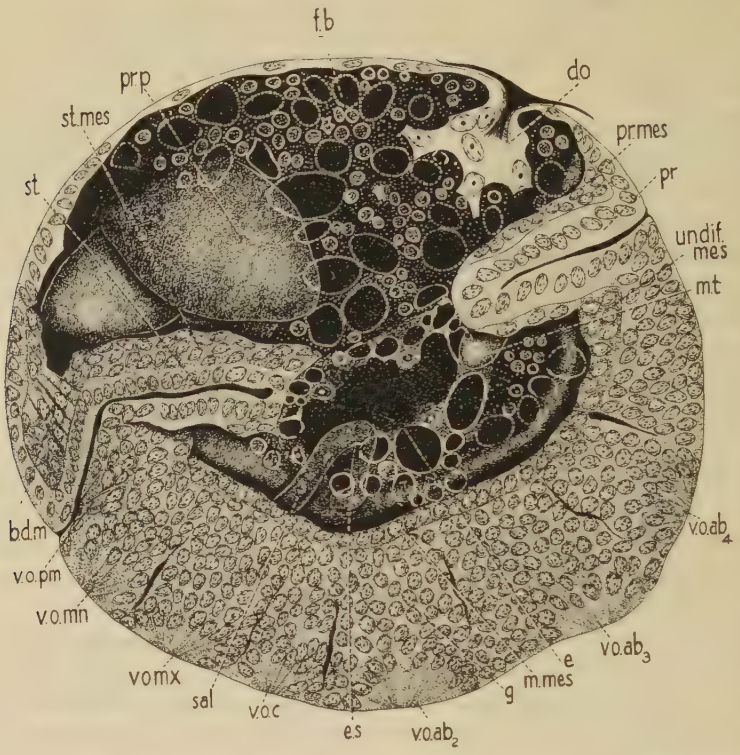
During the eighth day the inner blunt end of the stomodaeum comes into contact with the clump of yolk-laden endoderm in the middle of the egg (fig. 67D, Pl. 6). The mesodermal investment of the fore-gut has now greatly enlarged, and accumulates as a thick ridge of cells along its upper surface (Text-fig. 7).

In more advanced embryos this dorsal heaping up of mesoderm again diminishes, for many of its cells migrate on to the wall of the mid-gut. Others spread down to invest the lateral and ventral walls of the fore-gut (fig. 110, Pl. 9). This complete investment of the fore-gut by mesoderm is found chiefly towards its hinder end, and provides the cells from which the large oesophageal dilator muscles will develop. The formation of these is described in section 15 (*b*) (i). Only a small part of the mesoderm remains on the fore-gut itself, and out of it will develop, in the late pupa, the feeble oesophageal musculature.

In the advanced embryo the inner tip of the fore-gut is sometimes seen intruding, as an oesophageal valve, into the cavity of the mid-gut (Text-fig. 8). Even in pupae the hind end of the oesophagus is still closed (Text-fig. 9); it does not, indeed, seem to open into the mid-gut cavity till shortly before the larva emerges.

(ii) *The Hind-gut.* In most, though not all, embryos the proctodaeum arises a little later than the stomodaeum. Like the latter, it very soon develops a lumen, and acquires an investing sheath from the adjacent mesoderm (Text-fig. 5; fig. 58A, Pl. 5).

By the ninth day its blind tip has come into contact with the central yolk-laden mass of endoderm (Text-fig. 7). Hitherto it has remained a simple



TEXT-FIG. 7. Nine-day embryo bisected (see footnote to Text-fig. 5). The section is taken slightly to the side of the ventral mid-line, in order to show the 'ventral organs'. The stomodaeum and proctodaeum have grown in length, and have come in contact with the endoderm, which is now sharply delimited from the rest of the mass of yolk-cells (fat-body). The somites, which by this time are disrupting, are not visible, for they lie to the side of the row of developing nerve-ganglia; the anal and fifth abdominal somites have not yet formed. The maxillary (salivary) gland is conspicuous, and shows the beginning of an 'end-sac'. The Malpighian tubes are forming. The 'dorsal organ' is at the height of its development. 'Ventral organs' are shown from the pre-mandibular to the fourth abdominal segment. The overlying ganglia are becoming larger, and successive ganglia are already partly fused.

Lettering. *b.d.m* developing buccal dilator muscles; *d.o* 'dorsal organ'; *e* endoderm; *e.s* 'end sac' of salivary gland; *f.b* fat-body; *g* ganglionic tissue; *m.mes* median mesoderm; *m.t* Malpighian tube; *mes.an* mesoderm of anal segment; *pr* proctodaeum; *pr.mes* proctodaeal mesoderm; *pr.p* posterior lobe of protocerebrum; *sal* salivary gland; *st* stomodaeum; *st.mes* stomodaeal mesoderm; *undif.mes* mesoderm not yet differentiated into somites; *v.o.ab*$_{2-4}$, *v.o.c*, *v.o.mn*, *v.o.mx*, *v.o.pm*, 'ventral organs' of second to fourth abdominal, collum, mandibular, maxillary, and pre-mandibular segments respectively.

undifferentiated ingrowth, with narrow lumen, and with a single layered investment of mesodermal cells (fig. 83, Pl. 7; fig. 103, Pl. 9). But now signs of differentiation appear within it, the lumen becoming wider, and a terminal rectal chamber being seen in process of development. The mesodermal investment has now increased in thickness (fig. 113, Pl. 9).

In the advanced embryo the 'rectal valve' (Text-fig. 8) appears. If we compare Text-fig. 8 with fig. 113, Pl. 9, we see that the rectal valve does not arise as an ingrowth of the hinder end of the colon into the rectum, as might have been expected, but that it is a differentiation within the terminal chamber itself; for the walls of this chamber are two cell-layers in thickness, of which the inner layer, by separation, forms the valve.

Subsequently cells from the investing mesoderm spread down over the enlarging rectum, and are probably the main source of its powerful musculature. Whether additional cells are derived from the disruption of the anal somites is uncertain.

The hind-gut does not acquire an opening into the mid-gut cavity until shortly before the larva emerges.

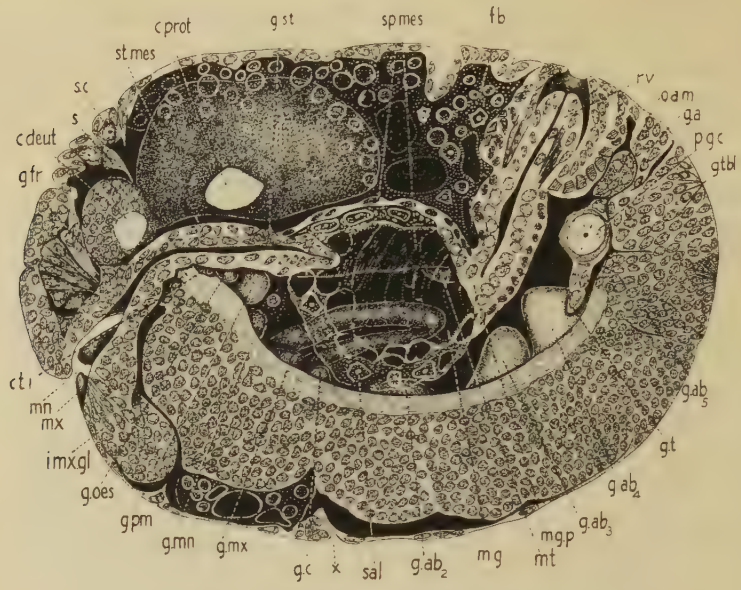
(iii) *The Mid-gut.* The mid-gut epithelium develops chiefly out of the yolk-laden endoderm cells of the gastrula. The development of the endoderm has been described in section 2.

By the time the germ-band is forming, the endoderm cells have usually begun to acquire a richer content of cytoplasm, and therewith the yolk begins gradually to disappear. Like the partitions between the yolk-pyramids, the membrane delimiting the endoderm has now also broken down, and the cytoplasm of the latter seems to be directly continuous with that which enmeshes the remainder of the yolk (Text-fig. 5; fig. 52, Pl. 5). Not more than two nuclei are present within the endoderm; they are unusually large with prominent nucleoli, and are easily distinguishable from the nuclei of the 'yolk-cells'. They generally lie closely together, and therefore often appear in one and the same section (fig. 58 B, Pl. 5).

The cytoplasm of the endoderm cells is, at this stage, often seen to be crowded with small, rounded or rod-shaped inclusions, staining weakly with haematoxylin (fig. 54, Pl. 5). Their nature is uncertain; they do not seem to be parasitic organisms. I have never seen them in later embryos.

During the next few days the yolk continues to disappear from the endoderm cells. But the latter multiply only very slowly, and even by the eighth day not more than five or six can be counted. With increase in number they decrease in size, and come to resemble more and more closely the adjacent 'yolk-nuclei'. Their cytoplasm does not appreciably increase in quantity, and they form a loose reticulum of cells without detectable cell-walls.

During the ninth day the endoderm cells, now about ten in number, shrink from one another, and so come to enclose a spacious but irregular cavity within the middle of the yolk. This is the lumen of the future mid-gut (Text-fig. 7; figs. 103, 104 A, Pl. 9). Its limits are usually difficult to make out, for in the adjacent yolk large and irregular vacuoles are also present; and, moreover, the mid-gut cells can no longer be distinguished with certainty from yolk-cells which may lie in the neighbourhood. With this rather vaguely outlined mid-gut Anlage the tips of the stomodaeum and proctodaeum are now in contact.

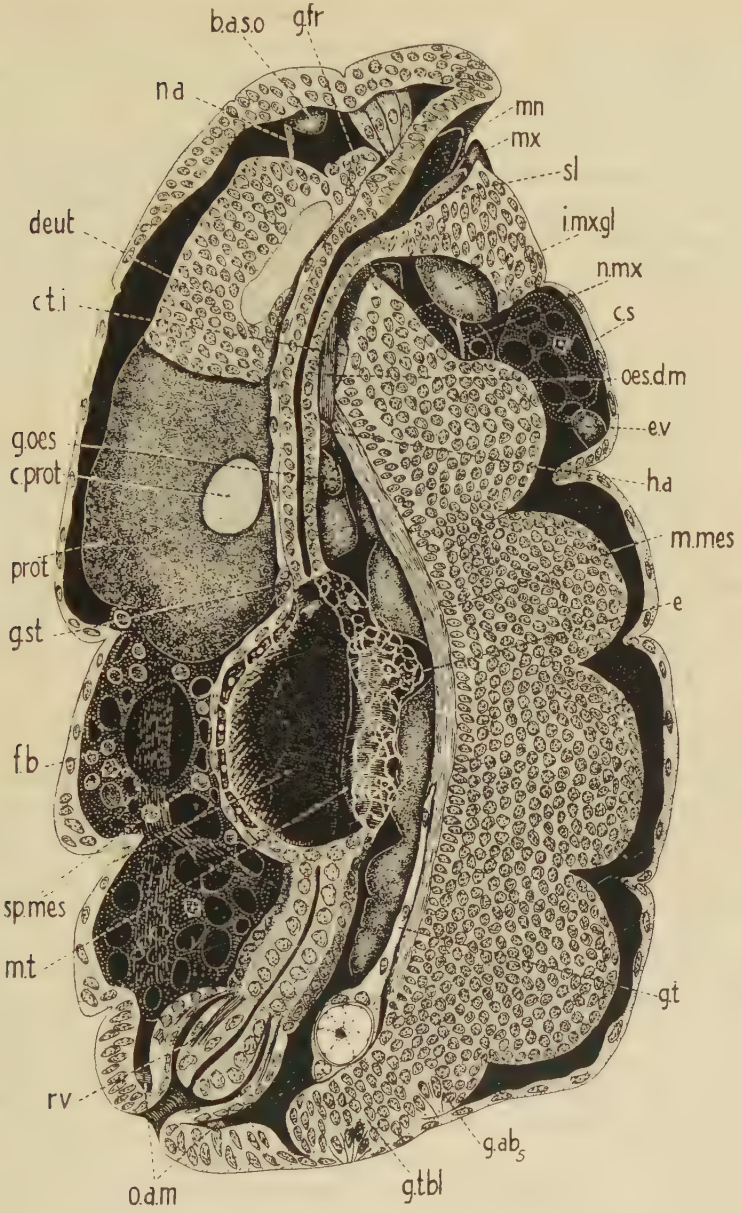


TEXT-FIG. 8. Advanced embryo, bisected. (See footnote to Text-fig. 5.)

The body-wall has now been completed; note intersegmental grooves on mid-dorsal surface. The endoderm is now roofed over by splanchnic mesoderm; the endoderm cells, still highly vacuolated, are in process of forming a mid-gut, into whose cavity the fore-gut intrudes as an oesophageal valve. In the hind-gut the rectum, with intruding rectal valve, has appeared. The yolk-cells that are excluded from the mid-gut are the developing fat-body. The salivary gland extends almost to the tip of the hind-gut. The intermaxillary gland is in process of development. Of the Malpighian tube only a small part is visible, for it lies to the side of the mid-gut. The genital tube has appeared and contains a single germ-cell. In the brain the protocerebral and deutocerebral ganglia, with commissures, are seen; only a fragment of the tritocerebral ganglion is visible, for most of it lies to the side of the oesophagus. The ganglia of the ventral nerve-cord have fused, the successive ganglia projecting up to the side of the neuropilem. A fifth abdominal ganglion has separated from the teloblastic ganglion, and is showing 'ventral organ' structure; a minute anal ganglion is apparently present. In the visceral nervous system the oesophageal ganglion and the developing frontal and stomachic ganglia are recognizable.

Lettering. c.deut deutocerebral commissure; *c.t.i* inferior tritocerebral commissure; *c.prot* protocerebral commissure; *f.b* fat-body; *g.a* anal ganglion; *g.ab* $_{2, 3, 4, 5}$ ganglia of the second to fifth abdominal segments; *g.c* ganglion of collum segment; *g.fr* frontal ganglion; *g.mn* mandibular ganglion; *g.mx* maxillary ganglion; *g.oes* oesophageal ganglion; *g.pm* pre-mandibular (tritocerebral) ganglion; *g.st* stomachic ganglion; *g.t* rudiment of genital tube; *g.tbl* teloblastic ganglion; *i.mx.gl* intermaxillary gland; *m.g* mid-gut; *m.g.p* proctodaeal component of mid-gut epithelium; *mn* mandible; *m.t* Malpighian tube; *mx* maxilla; *o.a.m* developing occlusor ani muscle; *p.g.c* primordial germ cell; *r.v* rectal valve; *s* epidermal septum between right and left pre-antennary ganglia; *sal* salivary gland; *s.c* setigerous cell; *sp.mes* splanchnic mesoderm; *st.mes* stomodaeal mesoderm; *x* intersegmental groove delimiting posterior end of collum segment.

Meanwhile the mesoderm of the mid-gut has become evident. It arises in a most unusual manner, for it develops not from the somites, but from the mesoderm that lies heaped up along the dorsal wall of the stomodaeum. During the ninth day this mesoderm begins to grow backwards over the



TEXT-FIG. 9. Pupa, bisected. (See footnote to Text-fig. 5.)

The principal organs of the pupa show little advance over those depicted in Text-fig. 8. As in the latter figure, the splanchnic mesoderm has been drawn as an arching roof to the mid-gut, but to simplify the drawing no attempt has been made to indicate the lateral wall of mid-gut epithelium. Note the contrast between the very reticular endoderm cells on the floor of the mid-gut and the relatively firm endoderm cells that form its roof.

Lettering. *b.a.s.o* basal antennal sense organ; *c.prot* protocerebral commissure; *c.s* collum segment; *c.t.i* inferior tritocerebral commissure; *deut* deutocerebrum; *e* endoderm cells

mid-gut cells (fig. 103, Pl. 9), while at the same time it spreads down to invest them laterally; only the most ventrally situated mid-gut cells remain free from investing mesoderm. This arching roof of splanchnic mesoderm is shown in fig. 89, Pl. 7, and in Text-figs. 8 and 9.

The character of the mid-gut cells now begins to change (fig. 89, Pl. 7). They have increased to about twenty in number. Those mid-gut cells that lie beneath the investing layer of splanchnic mesoderm, and from which the roof and lateral walls of the mid-gut will develop, are now in process of forming a loose epithelium; the cytoplasm retains its feebly staining character, but is beginning to acquire a firmer texture, and it is now completely denuded of yolk. The floor of the mid-gut, on the other hand, is still constituted by large irregularly reticulate cells, within which yolk-grains are present, though in diminished numbers. Sometimes a degenerated yolk-laden cell may be present within the lumen of the mid-gut (fig. 103, Pl. 9).

In more advanced embryos the mid-gut epithelium acquires a firmer character, and therewith the distinction between floor- and roof-cells becomes very apparent (fig. 107, Pl. 9). In the roof-cells the cytoplasm is becoming darker and more dense, and the refringent concretions may already be present in considerable numbers. The floor-cells have lost their last remnant of yolk, and have merged into the general contour of the mid-gut wall. Their cytoplasm is still highly reticular and feebly staining, and they are devoid of concretions. There is no splanchnic mesoderm associated with them. In this condition the mid-gut epithelium remains until well into the pupal period (fig. 108, Pl. 9).

In the late pupa the development of the mid-gut wall is completed. The roof-cells enlarge, their nuclei acquire large nucleoli, and a striated border appears. The floor-cells, on the other hand, remain free from any trace of concretions; nor does a striated border form. The splanchnic mesoderm now completely invests the mid-gut, having spread under the floor-cells (fig. 109, Pl. 9).

The hindermost end of the mid-gut wall has an entirely different origin. In the fully developed intestine this terminal portion of the mid-gut resembles the most anterior portion of the hind-gut (Text-fig. 6 C), pointing therefore to a probable derivation from the proctodaeum of the embryo, and not from endoderm cells. This is readily confirmed when embryos after about the ninth day are examined. In fig. 113, Pl. 9, for example, may be seen the participation of the most anterior part of the proctodaeum in the formation of the mid-gut wall, the distinction between its cells, and the faintly staining

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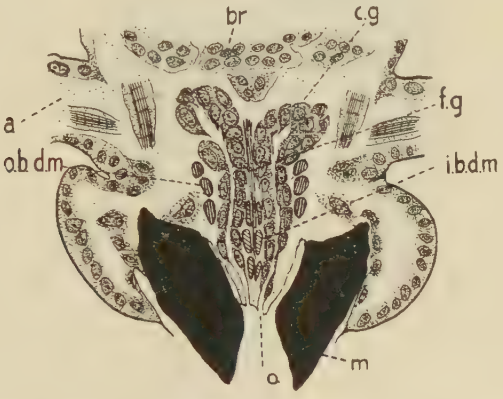
on floor of mid-gut; *e.v* exsertile vesicle; *f.b* fat-body; *g.ab*$_5$ fifth abdominal ganglion; *g.fr* frontal ganglion; *g.oes* oesophageal ganglion; *g.st* stomachic ganglion; *g.t* genital tube; *g.tbl* teloblastic ganglion; *h.a* hind tip of hypopharyngeal apophysis, bending round the circum-oesophageal connective; *i.mx.gl* intermaxillary gland; *m.mes* median mesoderm (this now forms the median band of neuroglial tissue); *m.t* Malpighian tube; *mn* mandible; *mx* maxilla; *n.a* antennal nerve; *n.mx* maxillary nerve; *o.a.m* occlusor ani muscle; *oes.d.m* oesophageal dilator muscle; *prot* protocerebrum; *r.v* rectal valve; *sl* superlingua; *sp.mes* splanchnic mesoderm of mid-gut.

reticular endoderm cells, being very evident. The proctodaeal component of the mid-gut epithelium is seen also in Text-fig. 8 and fig. 114, Pl. 9. The incorporation of some proctodaeal cells into the mid-gut wall is well known for some insects, where it may have the effect of drawing even the orifices of the Malpighian tubes into the mid-gut.

(iv) *The Malpighian Tubes.* These arise, during the ninth day, as a pair of outgrowths from the anterior end of the proctodaeum, and are at first without a lumen (figs. 106, 113, Pl. 9). They grow forward on either side of the



TEXT-FIG. 10. Clypeal glands. The drawing represents a frontal section of the head of a second instar larva, and shows the glands for their entire length.

Lettering. *a* base of antenna; *br* brain; *c.g* clypeal gland; *f.g* fragment of frontal, (visceral) ganglion: the greater part of the ganglion lies dorsal to the section; *i.b.d.m* inner buccal dilator muscle; *m* mandible; *o* orifice of clypeal gland; *o.b.d.m* outer buccal dilator muscle.

developing mid-gut, and in the late embryo extend almost the full length of the latter. By this time a lumen has appeared within them (figs. 107, 108, Pl. 9). In the relatively large size and texture of cytoplasm of their cells they present, at this time, the appearance of normal developing Malpighian tubes.

Early in the pupal period we see the first sign of the differentiation of the Malpighian tubes into a terminal part of more crowded darker cells, and a middle part in which the cells are already paler, with more widely dispersed nuclei, while at the proximal end of the tube a region with rather more crowded nuclei is seen, representing the zone of future cell-proliferation (fig. 114, Pl. 9). By the end of the pupal period the distinction has become more marked (fig. 133, Pl. 10).

The retrogression of the Malpighian tubes falls within the larval period; see Post-emb. Dev., section 5.

10. *The Glands*

(i) *The Clypeal Glands* (Text-figs. 10, 16). These glands have not hitherto been described in *Pauropus*. They lie on the floor of the clypeus, between the

inner and outer rows of buccal dilator muscles (Text-fig. 18F). They are very simple glands, with a narrow unbranched lumen, and open, in front, on to the roof of the pre-oral cavity. Their hinder ends do not extend farther back than the bases of the antennae. Their cells are small, and without a strikingly glandular texture of cytoplasm, their secretory function being inferred from the presence of their ducts which lead into the pre-oral cavity.

TEXT-FIG. 11. Pre-mandibular Gland; from an animal cut in sagittal section.
Lettering. d duct.

They are of ectodermal origin. In embryos aged about ten days, sections taken parallel with the floor of the clypeus display these glands as a pair of elongate columns of cells, without lumen, in process of growing backward to the side of the frontal ganglion (fig. 91, Pl. 7). The suspicion that they are ectodermal glands is at once confirmed in sections through rather less advanced embryos, where they are seen in course of development as ingrowths of cells from the roof of the pre-oral cavity (fig. 90, Pl. 7). Even in very advanced embryos a lumen is not recognizable (fig. 102, Pl. 9), and this does not seem to develop until late in the pupal period.

Both in diplopods (Reinecke, 1910) and chilopods (Fahlander, 1938), glands opening into the roof of the 'buccal cavity' have been described. They are more complex in structure than the clypeal glands of *Pauropus*. In *Scolopendra*, according to Heymons (1901), they are ectodermal ingrowths.

(ii) *The Pre-mandibular Glands* (Text-figs. 11, 16). These are a pair of relatively large glands, located dorso-laterally in the second abdominal

segment. They have already been described by Schmidt (1895) as salivary glands, and by Silvestri (1902) under the name of 'bucal gland'. Each is a compact mass of glandular tissue, and is devoid of a lumen. In addition to the large functional gland-cells, we can often distinguish, in the glands, a central band of much smaller cells, with diminutive and deeply staining nuclei. These smaller cells are presumably a reserve from which effete gland cells are replaced; for in some cases they are not present at all; and in others, as in the example shown in Text-fig. 11, apparently transitional stages are present, in which certain large gland-cells are found with nuclei of the diminutive kind. The ducts are remarkably fine tubes, with about six fusiform nuclei along their length; they closely resemble tracheae, and were indeed referred to as such by Schmidt, who did not observe their connexion with the glands. This connexion is with the lowest part of the gland, but they are not continued into the substance of the latter. Their orifices are in a most unexpected position, being lateral to the bases of the mandibles (Text-fig. 12). The cavity into which they drain is not the pre-oral cavity proper, but a pre-oral cavity enlarged by the inturning of the lateral margins of the clypeus (cf. section 6 (ii) (*b*)).

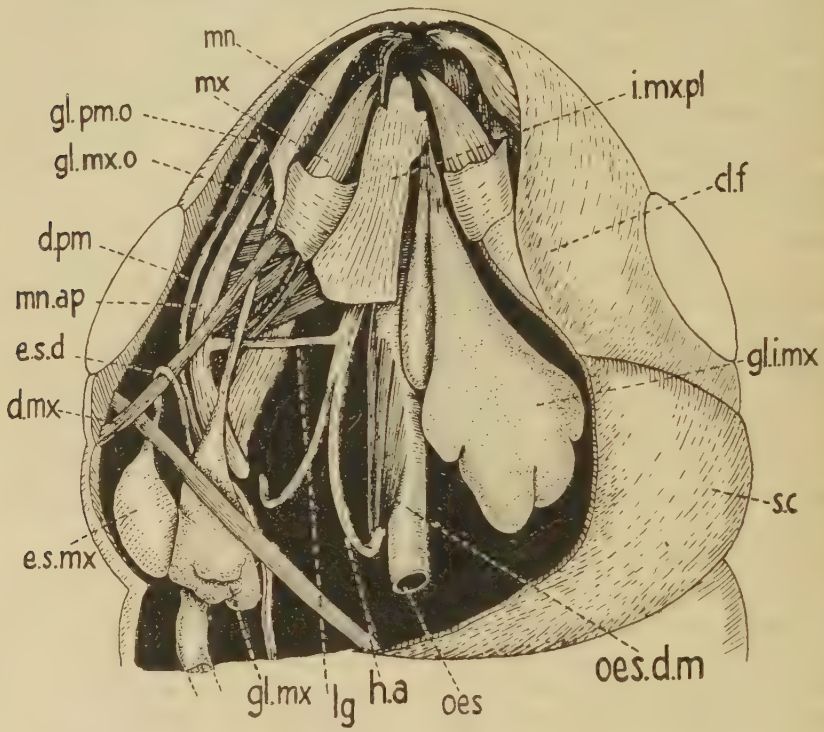
The pre-mandibular glands form out of the pre-mandibular somites. In section 8 (iii) has been given an account of the early phases of their development, up to the stage in which the somites have become converted into the recognizable rudiments of a pair of tubular glands. It now remains to describe the final phases of their development.

In late embryos, owing to the change in form which the head has undergone, the gland rudiments are best seen in frontal sections of the head, in which they may appear for their entire length in a single section. Fig. 111, Pl. 9, is a drawing of such a section, and shows the developing gland, to the side of the brain, extending deeply into the head from its point of attachment to the ectoderm just in front of the mandible. The gland is shown also in fig. 116 B, Pl. 10. It is, at this period, still a long cord of cells, but a lumen is no longer recognizable.

In the advanced embryo the position of the epidermal attachment (future orifice) of the gland undergoes displacement from a position antero-median to the mandible into a position which is actually lateral to the mandible. This will readily be seen by comparing fig. 111, Pl. 9, with fig. 119, Pl. 10, the latter figure representing a section cut approximately 'horizontally' along the head of an early pupa. The displacement seems to be associated with the development of the clypeal folds, and its character can be more readily visualized if the topography of the head of an advanced embryo, as shown, for example, in fig. 30 B, Pl. 3, be kept in mind. Much of the inferior surface of the clypeus is composed of pre-mandibular ectoderm (see section 6 (ii) (*b*)), and the downgrowth of the epidermis on to the side of the head will clearly have the effect of drawing the gland-attachment across the front of the base of the mandible into its position to the side of the latter.

In early pupae we see the first sign of histological differentiation of the gland, for cells with large and with small nuclei are now distinguishable (fig. 119,

Pl. 10). In later pupae the gland moves farther back into the second abdominal segment (fig. 122, Pl. 10), this backward displacement being probably due to pressure from the elongating mandibular apodeme (cf. fig. 120, Pl. 10), and perhaps also to enlargement of the brain and muscles of the head. At the



TEXT-FIG. 12. View, from below, of interior of head, to show cephalic glands and their ducts. Of the pre-mandibular gland, only the duct is shown.

Lettering. *cl.f* clypeal fold; *d.mx* depressor muscle of maxilla; *d.pm* duct of pre-mandibular gland; *e.s.d* duct of end-sac; *e.s.mx* end-sac of maxillary gland; *gl.i.mx* intermaxillary gland; *gl.mx* maxillary gland (only its most anterior end shown); *gl.mx.o* orifice of duct of maxillary gland; *gl.pm.o* orifice of duct of pre-mandibular gland; *h.a* hypopharyngeal apophysis; *i.mx.pl* intermaxillary plate; *lg* fibrous ligament from mandibular apodeme to hypopharyngeal apophysis; *mn* mandible; *mn.ap* mandibular apodeme; *mx* maxilla; *oes* oesophagus; *oes.d.m* oesophageal dilator muscle; *s.c* collum segment.

same time the hitherto elongate form of the gland is lost, and it assumes the compact clumped condition of the definitive organ.

The duct of the gland is now also in course of formation. It seems to be of epidermal and not mesodermal origin; but critical observations on this point are difficult to make. In early pupae the epidermal attachment of the gland is in the angle between the mandible and the clypeal fold (fig. 119, Pl. 10), and it lies immediately below the elongating mandibular apodeme, which is therefore not present in the same 'horizontally' cut section. When, in rather later

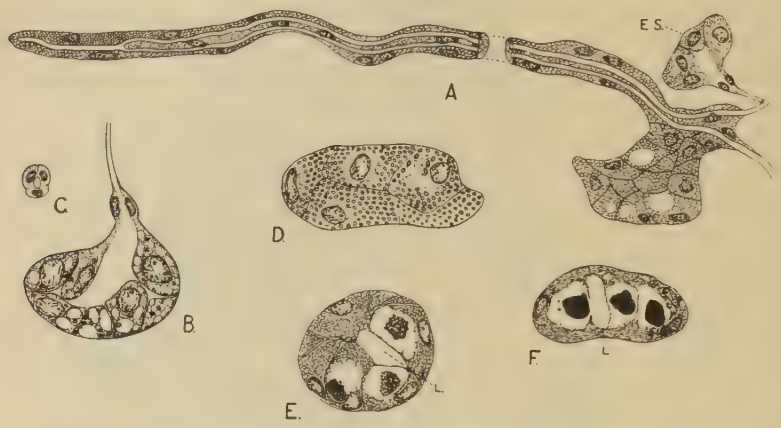
pupae, the inner wall of the clypeal fold in the neighbourhood of the gland-attachment is examined, its cells appear markedly fusiform, with elongate nuclei (fig. 121, Pl. 10). In still later pupae, when the gland has moved back into the second abdominal segment, the duct is seen for the first time, and it is cells of this latter type out of which it is constructed. Fig. 122, Pl. 10, has been drawn to illustrate this point, and evidently suggests an epidermal rather than mesodermal origin for the duct.

Wheeler, as long ago as 1893, described in the embryo of *Xiphidium* (Orthoptera) the peculiar 'sub-oesophageal' bodies which have since been observed in many other insect embryos. Although there is still some dispute as to their origin in the embryo, weight of evidence favours their derivation from the pre-mandibular mesoderm. In the embryo of *Scolopendra* there is found, in their place, some transient 'lymphoid tissue' (Heymons, 1901). Wheeler was the first to suggest a derivation of the sub-oesophageal bodies of insects from an excretory gland, and was led to compare them with the 'green gland' of Malacostraca. Heymons expressed a similar idea: 'The possibility may perhaps not be wholly excluded, that in it (lymphoid tissue) we have the modified remains of a kind of nephridium or primitive cephalic excretory gland.' These predictions have been fulfilled by the discovery, in the embryo of Symphyla, of a large pre-mandibular segmental organ, in which the end-sac assumes the form of a tubular nephrocytic organ (Tiegs, 1940); but here the gland disrupts shortly after the larva leaves the egg, and only the associated nephrocytic organ survives. In *Pauropus*, on the other hand, the gland remains even in the adult animal. It is surprising, however, to find that here its tubular character is completely obscured as development proceeds.

Wheeler, who first described the sub-oesophageal bodies in insect embryos, does not seem to have recognized the resemblance of their cells to nephrocytes, though his drawings plainly reveal it. More recent writers have compared them with the 'pericardial cells', and have attributed to them some excretory function (Heymons, 1895; Strindberg, 1916; Wiesmann, 1926; Mansour, 1927). They are generally believed to be restricted to the embryonic and early larval phases of the insect. In *Calandra oryzae*, however, they survive throughout the larval period, when they can be seen attached to the undersurface of the mycetoma, and can be recognized, though in diminished size, even in the imago (Tiegs and Murray, 1938). In this insect they undoubtedly resemble nephrocytes. To test the matter a number of larvae, pre-pupae, pupae, and adult weevils were injected by means of a micropipette with ammonia carmine, the injections being kindly made for me by Dr. F. H. Drummond. The insects were killed 24–48 hours after injection, fixed in formalin, and examined in sections. In every case the cells of the sub-oesophageal body were found to have absorbed the stain, and with an intensity in no way inferior to that of the nephrocytic cells alongside the heart.

In *Pauropus* the organ must be a salivary gland, for it is not provided with an 'end-sac', and, moreover, does not show any nephrocytic activity towards trypan blue when this dye is injected into the blood (see further, below:

maxillary gland). In *Peripatus* the segmental organ of the equivalent (third) segment is also a salivary gland, though here an 'end-sac' is present. I previously referred to this gland in Symphyla as the remains of an ancestral excretory gland, since its orifice does not lie within the pre-oral cavity; but it now seems more likely that it is the remains of an ancestral salivary gland, which has survived in *Peripatus* and *Pauropus*, its retention in the latter having



TEXT-FIG. 13. Salivary (Maxillary) Gland.

A. Optical section along gland; exit duct to right. B. Section through 'end-sac', and part of its duct. C. Transverse section through duct of 'end-sac', close to its exit from the latter. D. Section through an 'end-sac', in which the cells are unusually heavily laden with brown granules. E. Transverse section through an 'end-sac', in which the granules are aggregating into clumps. E. Final stage of aggregation of granules into compact brown clumps; to the left one of these clumps appears to be undergoing extrusion from its vacuole into the lumen of the 'end-sac'.

Lettering. *e.s* 'end-sac'; *l* its lumen.

perhaps been made possible by the enlargement of the pre-oral cavity due to formation of the clypeal folds.

(iii) *The* (*Maxillary*) *Salivary Glands* (Text-fig. 13). These glands, which are the largest of the salivary glands, have already been described by Silvestri (1902) under the name of 'mandibular glands'. They are a pair of long tubular glands, traversed for their complete length by a narrow channel that doubles on itself near their hinder ends, to terminate each in an 'end-sac' in the collum segment (Text-fig. 13 A). From near the anterior end of each, just below the 'end-sac', a large mass of glandular tissue depends on the floor of the collum segment. The gland-cells throughout the length of the glands are comparatively large, with finely reticular cytoplasm.

The exit ducts from the glands are a pair of narrow channels, scarcely wider than the ducts from the pre-mandibular glands, and with only sparsely distributed nuclei in their walls. They enter the head and, bending downward, pass forward median to the maxillae, to open into the pre-oral cavity just behind the bases of the mandibles (Text-fig. 12).

No description of the 'end-sacs' has hitherto been given. They are a pair of small, rounded vesicles, not more than 0·03 mm. in diameter, and are situated dorso-laterally in the collum segment, just above the large masses of salivary gland tissue that occupy much of its floor. From each 'end-sac' the narrow end-sac duct passes forward into the head, bends round the depressor muscle of the maxilla, and enters the glandular tissue (Text-figs. 12, 13 A). Shortly beyond its exit from the end-sac each duct shows a small swelling, within which three (rarely four) nuclei are lodged (Text-fig. 13 B, C). Although contractile fibrils are not visible within the cells, it seems probable that they exert a sphincter action on the end-sac.

The presence of the end-sacs first became apparent in animals that had been injected with trypan blue, as a test for the presence of nephrocytic tissue in *Pauropus*. The injections, which were made for me by Mr. A. M. Clark by the use of a Peterfi micromanipulator, were performed on animals lightly anaesthetized with ether, a small quantity of a 1 per cent. trypan blue solution being introduced through one of the posterior tergites. As might be expected with such fragile animals, the mortality was high; five animals, however, survived, and on these the present account is based. Within an hour after injection of the dye the two end-sacs can be seen, through the transparent cuticle of the living animal, tinged pale blue, and thereafter the colour gradually deepens to an intense blue. Fixed preparations of animals killed 3–17 hours after injection show the dye within the substance of the end-sac. None of the other glands, nor the Malpighian tubes, are affected; nor are there any scattered nephrocytes in *Pauropus* comparable with the 'pericardial cells' of insects.

In section the end-sac is seen to be composed of relatively large uninucleate cells, with clefts from the central cavity of the vesicle extending between them (Text-fig. 13 B). The cytoplasm commonly shows a vacuolated and faintly fibrillar texture, and brownish granules may be present either in relatively small numbers (Text-fig. 13 B), or in great abundance (Text-fig. 13 D). It is upon the granules that the trypan blue is absorbed. Sometimes end-sacs are encountered in which the granules from the cytoplasm are aggregated into large clumps, which are then lodged within spacious vacuoles in the cells (Text-fig. 13 E). Within these vacuoles the granules then fuse into compact brownish masses, which are thereafter discharged into the cavity of the end-sac (Text-fig. 13 F). These are processes with which we are already familiar in the end-sacs of certain cephalic glands in Crustacea (Burian and Muth, 1924) and tracheates (Bruntz; see summary by Ehrenberg, 1924).

In the embryo the salivary glands develop out of the somites of the maxillary segment. The earlier phase of this development has already been described above (section 8 (v)).

The end-sacs are first seen in embryos at about the time that the proximal ends of the glands become separated from the vestiges of the original coelomic sacs, which then become resolved into myoblasts for the developing maxillae. The glands have, by this time, become completely doubled on themselves

(Text-fig. 7), the hindermost tips of the bent tubes now lying well within the collum segment, whence they extend forward to their epidermal attachment on the median aspect of the maxilla immediately to the rear of the mandibles. In each gland the rudiment of the end-sac is recognizable as a pronounced swelling at its blind tip, the cells being already distinguishable by their larger size (fig. 89, Pl. 7; fig. 117, Pl. 10).

In the advanced embryo the maxillary glands extend well back in the abdomen, where they are now seen lying to the side of the mid-gut (Text-fig. 8). In the pupa they attain their mature character, the two closely apposed limbs of the completely bent glands fusing with one another, the ducts remaining, however, apart. Their cells enlarge, and acquire a typical glandular texture. The end-sacs become withdrawn into the collum segment.

The origin of the exit ducts is difficult to determine; as far as I have been able to make out, these come from the epidermis and not from the gland-Anlage. In early pupae the anterior tip of each of the maxillary glands still lies well within the head, at the base of the maxilla, where it is connected with the epidermis on the median aspect of the maxilla just behind the mandible (fig. 123, Pl. 10). In later pupae the glandular tissue has receded into the collum segment, its connexion with the base of the maxilla being by a just perceptible string of narrow cells, whose elongate nuclei and deficient cytoplasm recall those of the epidermis at the former gland-attachment, and not of the gland-Anlage (cf. figs. 123 and 124, Pl. 10).

Maxillary glands, furnished with 'end-sacs' and mesodermal in origin, are known in Diplopoda and Symphyla. In Fahlander's recent work (1938) a maxillary gland with 'end-sac' has been described in *Scutigera* and *Lithobius*, but its presumed mesodermal origin still requires proof. In *Scolopendra*, where the development of the cephalic glands has been examined, mesodermal glands are absent (Heymons, 1901).

(iv) *The Intermaxillary Glands* (Text-figs. 12, 16 B, 17). These have already been described by Silvestri (1902) for *Allopauropus brevisetus* under the name 'maxillary gland'. In *Pauropus silvaticus* their structure is more complex than might have been expected from Silvestri's description.

They comprise a pair of large bilobed masses of glandular tissue, often closely apposed, and sometimes even fused into a single compact mass. They occupy part of the floor of the head-capsule, whence they bend down on to the floor of the collum segment. The glandular tissue consists of large irregularly vacuolated cells, with large nuclei located at the periphery. But there are also sometimes present groups of cells with smaller nuclei, from which, possibly, effete glandular cells are replaced. From the glandular tissue several irregular spacious channels pass forward, opening between the maxillae and the intermaxillary plate. The glands are presumably salivary in function, and with trypan blue give no sign of nephrocytic action. They are innervated from the sub-oesophageal ganglion by a branch of the maxillary nerve (Text-fig. 17).

The intermaxillary glands form from the ectoderm of the maxillary segment. Their development takes place in an unexpected manner, for they

arise from cells which might have been regarded as prospective ganglion cells. If, in a 10-day embryo, a section is cut transversely through the hinder part of the maxillary segment, the 'ventral organs' of that segment are found to be associated with a paired mass of cells, which is itself moulded into the contour of the hinder part of the maxillary ganglion, but is in process of separating away from the latter. This is the rudiment of the intermaxillary gland. Fig. 98, Pl. 8, in which it is shown, is drawn from an embryo in which the entire gland-Anlage, even though not completely demarcated from the ganglion, is already distinguishable from the latter by the character of its nuclei, a distinction which is, however, not always visible.

In more advanced embryos, in which the mandibular sternum has become invaginated into the pre-oral cavity, the sternum of the maxillary segment remains as the developing intermaxillary plate (cf. fig. 30 A, Pl. 3). A section directed just above the floor of this segment is shown in fig. 94 E, Pl. 8. If this is compared with fig. 98, Pl. 8, it will be seen that the maxillary 'ventral organs' have now come closely together, and form most of the tip of the intermaxillary plate; internal to them is the developing intermaxillary gland, this being now completely separated by a cleft from the ganglion. With the change in position of the maxillary segment that has attended the enlargement of the pre-oral cavity, the intermaxillary gland-Anlage, hitherto fitting against the hinder wall of the ganglion, is brought into a position ventral to the latter (fig. 99, Pl. 8; Text-fig. 8).

Differentiation into the definitive gland now sets in. In fig. 94 E, Pl. 8, there may already be seen a tendency for the paired gland-Anlage to diverge beyond the limits of the maxillary ganglion. In more advanced embryos this divergence becomes more pronounced, while at the same time a second pair of lobes arises from each gland-Anlage (fig. 102, Pl. 9, fig. 123, Pl. 9). Within each gland the nuclei now tend to congregate around its margin, and an ill-defined lumen appears. Thereafter the simple flask-shaped character of the adult gland is attained. The cytoplasm of the cells does not assume its peculiar texture until shortly before the larva emerges from the pupa. The associated 'ventral organs' cease to be recognizable in the advanced embryo, their cells being partly incorporated into the epidermis of the intermaxillary plate and partly into the substance of the glands.

(v) *The Pseudocular Glands.* Under this name I refer to a pair of glands, by no means inconspicuous, that lie flattened out against the whole of the epithelium of the pseudoculi, lateral to the ingrown apodemes of the mandibles. They are shown in Text-figs. 16 A and 20. They are composed of large cells, with obviously glandular texture of cytoplasm, and are devoid of a duct. Their function is unknown.

The pseudocular glands develop from ectoderm. The cells from which they take origin are first distinguishable in advanced embryos, shortly after the protocerebral lobes of the developing brain have separated away from the lateral epidermis of the head. Among the normal epidermal cells certain enlarged cells are seen (figs. 111, 115 A, Pl. 9), and these soon become apparent

as the developing gland cells. Other enlarged epidermal cells, indistinguishable from those which are destined to become the gland cells, appear close by in the epidermis (fig. 111, Pl. 9); these are the setigerous cells referred to in section 14 (i).

In later embryos the prospective gland-cells separate out from the epidermis and begin to congregate between it and the tip of the ingrown mandible (fig. 100, Pl. 8). They are now much enlarged, obviously glandular in structure, and are plainly recognizable as the pseudocular gland.

It is possible that the pseudocular glands of *Pauropus* are to be compared with the 'cerebral glands' (Gehirndrüsen) of Chilopoda, to which Fahlander (1938) has recently drawn attention. Heymons (1901) long ago described the development of these glands out of the lateral epidermis of the head (*Scolopendra*) and compared them with the post-antennal organs of Tömösvary from other myriapods. Fahlander, however, points out that in the anamorphic chilopods, organs of Tömösvary and cerebral glands coexist. In chilopods these glands are innervated from the protocerebrum; in *Pauropus* I have not been able to detect a nerve supply to the pseudocular glands.

11. *The Reproductive Organs*

In the newly hatched larva the reproductive organs are still in a very rudimentary condition (Text-fig. 26 A), and consist of a narrow string of cells, without perceptible lumen, extending from the fifth abdominal segment forward into the third. The rudiment is unpaired, and lies below the intestine in a median groove along the roof of the nerve-cord. Only a single primordial germ-cell is present, and this is embedded in the genital rudiment almost at its hinder tip in the fifth segment; it is distinguishable, as usual, by its large clear nucleus. There is no discernible difference between the sexes.

The genital rudiment develops out of the unsegmented 'median mesoderm' (q.v., section 7). In the fifth abdominal segment the mesoderm remains, up to the ninth day, as a broad sheet of cells, in which the delayed formation of somites is only just beginning. Even before these somites have formed, the single primordial germ-cell is usually, though not always, recognizable in the heaped-up mesoderm, being distinguishable from the surrounding cells by its larger size and by the character of its nucleus (figs. 84, 85, Pl. 7); but whether it has arisen *in situ*, or is an immigrant from some other part of the embryo, cannot, owing to its late differentiation, be determined.

Most of the heaped-up mesoderm is used in the formation of the fifth abdominal somites and the teloblastic mesoderm (see section 8); there remains only a small aggregation of 'median mesoderm' cells, and these form a closely fitting investment for the single germ-cell (fig. 86, Pl. 7). In the advanced embryo, cells from this investing layer grow forward medially along the row of developing ganglia (Text-fig. 8), and extend eventually just into the third abdominal segment (Text-fig. 9). Owing to the presence of the single germ-cell in the fifth segment, the genital tube is here at its widest. In this condition the genital rudiment survives into the first instar larva.

The simplicity and directness of development of the genital rudiment is remarkable. In *Peripatus* (Sedgwick, 1887), and in those myriapods that have hitherto been examined—*Julus* (Heathcote, 1888), *Scolopendra* (Heymons, 1901), *Hanseniella* (Tiegs, 1940)—the genital rudiment is paired and tubular, and the lumen of the genital tube is formed by the concrescence of the cavities of successive coelomic sacs. Even in *Julus*, where the genital rudiment is an unpaired tube lying beneath the mid-gut, as in *Pauropus*, its paired origin from the two rows of coelomic sacs has been proved (Heathcote). In *Pauropus*, on the other hand, it is not even a derivative of the somites, but is developed *in situ* in the unsegmented median mesoderm. The absence of any coelomoduct associated with the genital rudiment is also noteworthy.

In the comparatively late appearance of the primordial germ-cell within the mesoderm, *Pauropus* resembles other myriapods. In many insects, on the other hand, the germ-cells are set aside as the familiar 'polar cells' at the hind end of the blastoderm, whence they migrate into the coelomic sacs when these later develop. It may well be, as Heymons (1901) has suggested, that in myriapods they undergo a similar migration, which remains, however, undetected owing to delayed differentiation of the germ-cells. The hope that *Pauropus* might throw some light on this question has not been fulfilled; the solitary germ-cell is never distinguishable except within the mesoderm of the relatively late embryo.

12. *The Haemocoele, Fat-body, and Blood*

(*a*) *Adult Anatomy*. In adult animals with ample reserve material in the fat-body, and with ripe gonads, the haemocoele is, for the greater part, obliterated. But when the fat-body is depleted of its reserves, and when the reproductive organs are immature, quite a spacious haemocoele is revealed.

The fat-body is confined to the abdomen, its most anterior limit being the floor of the collum segment. When not laden with reserves, it is disposed mainly along the dorsal half of the body-cavity, there being also a thin, irregular, parietal layer which spreads down towards the bases of the legs, while there is also sometimes a thick layer beneath the nerve-cord. There is, therefore, a relatively spacious cavity to either side of the nerve-cord, these lateral neural blood-spaces (sinuses) communicating with one another by means of the epineural blood-space between the ganglionic chain and the floor of the intestine, and often also by spaces beneath the ganglia, when these are not obliterated by fat-body (fig. 109, Pl. 9). With the accumulation of reserves, the fat-body begins to encroach upon the lateral neural blood-spaces, vestiges of which are, however, usually present at the bases of the legs. The musculature of the leg-bases here acts as a barrier against the bulging fat-body; sometimes, however, the fat-body succeeds in insinuating itself between the muscles, and so enters the blood-spaces of the legs, and may, on occasion, spread even as far as the tibial segment. In the head, also, there is a blood-space, but it becomes much diminished in size by the strong development of head-muscles.

In minute structure the fat-body presents nothing unusual. It is a syncytial tissue, without clear evidence of any internal cell boundaries. Its nuclei are large, with prominent nucleoli, and the protoplasm is highly vacuolated, with the usual small spherical albuminoid inclusions in the protoplasmic reticulum. On occasion the fat-body may show a fine deposit of very minute crystalline concretions; this is, however, quite unusual and there is certainly no evidence of a continued accumulation of excretory products, such as we find in Symphyla, Collembola, and some other primitive insects (*Campodea*, *Japyx*). The fat-body shows clear evidence of phagocytic activity. To examine for the presence of phagocytic tissue in *Pauropus*, a number of animals were injected with a minute quantity of diluted India-ink, the injection being performed through one of the hinder tergal shields. The injections were made for me by Mr. A. M. Clark with a Peterfi micromanipulator. The animals were killed from 12 to 26 hours after injection, fixed in formalin, and then cut in sections. The mere presence of ink particles within the fat-body is not evidence of phagocytosis, for with such minute and fragile animals it is impossible to avoid forcing masses of ink particles directly into the cells at the site of injection. There is, however, clear evidence in my material of incorporation of granules of ink within fat-cells quite remote from the site of injection, as, for example, within the basal segments of the legs. True phagocytosis may be recognized also by the presence of India-ink particles exclusively within the protoplasmic reticulum of the fat-cells, and not within the vacuoles, although these comprise by far the greater part of this tissue. Examples of phagocytosis are shown in Text-fig. 14.

Very rarely cells having the appearance of blood cells are met with in sections of *Pauropus*. In my India-ink material I have never seen any sign of phagocytosis by such cells.

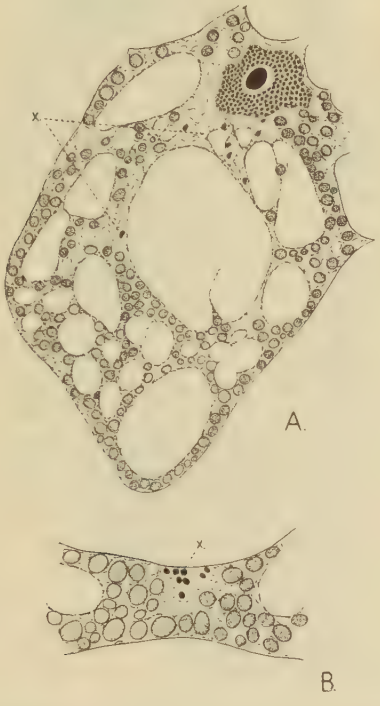
(*b*) *Development*. The fat-body arises from 'yolk-cells'. The early development of these has been described in section 4.

By the time the germ-band has appeared, from 50 to 80 large nuclei, with prominent nucleoli, can be counted in the yolk. There is a scarcely perceptible condensation of cytoplasm around them, this cytoplasm forming part of the delicate syncytial reticulum that pervades the interior of the egg and supports the yolk (figs. 53, 54, 58 A, B, Pl. 5; Text-fig. 5).

As the embryo develops, the yolk gradually disappears from the supporting reticulum, which is now recognizable as the fat-body in process of formation. It fills all the space between the developing intestine and the body-wall (Text-figs. 7, 8, 9; fig. 103, 107, Pl. 9), but in the late embryo and pupa shows a tendency to shrink away from the sides of the ganglion, so forming the lateral neural channels (fig. 108, Pl. 9). Spacious lateral neural channels are, however, not a constant feature of all pupae, and in some there is even an invasion of the sub-neural spaces by fat-body. The epineural sinus arises by withdrawal of yolk from between the mid-gut and the chain of ganglia (cf. fig. 108, Pl. 9). Throughout this period the cytoplasm of the fat-body has preserved its delicate reticular character, and even in the larva shows no clearly defined internal

cell-boundaries, nor have its nuclei shown any sign of division. In the newly hatched larva the fat-body may still contain a little yolk, but this is soon absorbed (Text-fig. 26 A).

At the hinder end of the abdomen the epineural blood-space has usually become fairly well defined in the late embryo, the fat-body shrinking away



TEXT-FIG. 14. Phagocytosis by fat-body. Injected particles of India-ink shown by *X*.
A. From an animal 26 hours after injection. B. After 12 hours.

from the space between the hind-gut and the ganglia (fig. 87, Pl. 7; fig. 106, Pl. 9).

After emergence of the larva all these blood-spaces become markedly enlarged, presumably owing to intake of water from without (cf. figs. 108 and 109, Pl. 9).

The blood-spaces of the head arise by withdrawal of the brain from the overlying epidermis (fig. 115, Pl. 9; figs. 116, 118, Pl. 10). With the enlargement of the brain and of the salivary glands during the pupal period, the fat-body is pushed back into the second abdominal segment, there being only a small amount on the floor of the collum segment (cf. Text-figs. 8 and 9).

I have not made any observations on the origin of the few problematical blood-cells.

The development of the fat-body of *Pauropus* out of the yolk-cells of the embryo is noteworthy; it is a feature that the Pauropoda share with the Symphyla, and, judging by Heathcote's fragmentary account (1888), with Diplopoda. In Chilopoda and insects, on the other hand, the entire mass of yolk becomes enclosed by mid-gut epithelium, and the fat-body develops instead from cells that are released from the walls of the somites.

13. *The Nervous System*

(*a*) *Structure of the Adult Nervous System.* Previous accounts of the nervous system are inadequate, and incorrect on important points. This applies especially to the brain and nerves that arise from it. The visceral nervous system has not hitherto been described.

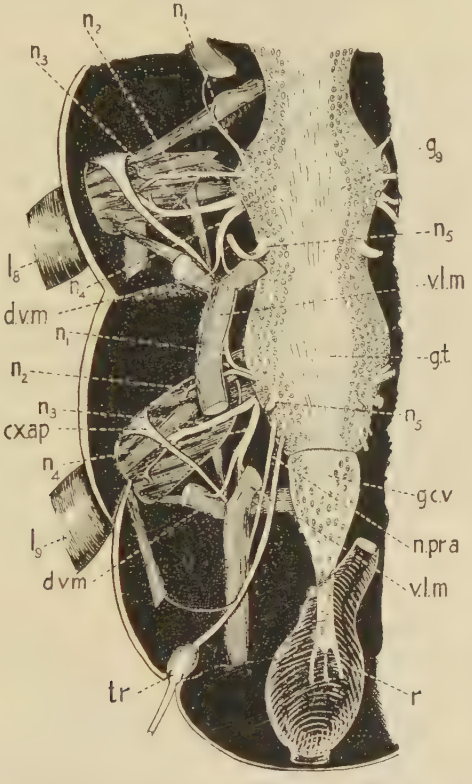
(i) *The Ventral Nerve-cord.* This consists of a small sub-oesophageal ganglion, formed by the fusion of the mandibular and a single maxillary ganglion, the fused ganglion being withdrawn from the head into the collum segment (Text-figs. 16 B, 17); of a single collum ganglion, partly fused with the former; and of a succession of nine large ganglia, one within each of the leg-bearing segments (Text-fig. 27 A). The connectives between successive ganglia are lightly clothed with nerve-cells.

From each abdominal ganglion arise five pairs of nerves (Text-fig. 15). The most anterior of these is clothed, for some distance from its base, with nerve-cells, and passes to some of the sternal muscles of the leg. Behind it arise three other nerves, of which the hindermost supplies the ventral longitudinal and dorso-ventral muscles, while the others pass down to the muscles of the leg. To the rear of these is a fifth nerve, which runs through the abdomen on to the dorsal body-wall, probably to supply the tergal muscles; it is joined in alternate segments by a large sensory nerve from each of the great sensory setae (trichobothria).

From the sub-oesophageal ganglia arise the mandibular and maxillary nerves; it is noteworthy that the succeeding pair of nerves are the nerves of the collum segment, there being no second maxillary nerve associated with the head. These nerves are shown in Text-fig. 17. The mandibular nerve passes forward alongside the ventral longitudinal muscle, turns sharply outward, and supplies the large muscles of the mandible, and probably also the anterior end of the ventral longitudinal muscle. The maxillary nerve runs forward below the mandibular nerve, and supplies the floor of the head. It is a surprisingly large nerve, most of its fibres passing direct to the large intermaxillary gland; I have not been able to detect the small branches that presumably go to the weak maxillary muscles.

The nerves from the collum ganglion recall those of the succeeding abdominal ganglia, except that the equivalent of the nerves to the leg-muscles is lacking. There is present, also, a long thin nerve attached to the lower end of the large head-levator muscle, that arises from the floor of the collum segment (Text-fig. 17).

The terminal ganglion (Text-fig. 15) is a composite ganglion; this is at once shown by the nerves to which it gives origin, for these supply not only the last leg-bearing segment, but also those to the rear of it.



TEXT-FIG. 15. Hind end of adult animal, showing terminal portion of ganglionic chain, exposed from above. On the left is shown the distribution of the nerves. To display the nerves to the legs, it has been found necessary to omit the overlying part of the ventral longitudinal muscle.

Lettering. *cx.ap* coxal apodeme; *d.v.m* dorso-ventral muscle; *g.c.v* caudal visceral ganglion; g_9 ganglion of ninth abdominal segment; *g.t* terminal (composite) ganglion; l_8, l_9 eighth and ninth legs; n_{1-5} five pairs of segmental nerves; *n.pr.a* nerves passing from terminal ganglion into pre-anal segment; *r* rectum; *tr* trichobothrium; *v.l.m* ventral longitudinal muscle.

Both the nerve-cord and its segmental nerves are invested by a 'neurilemmal sheath'. This membrane is generally difficult to detect, its nuclei, which lie flattened out against the ganglia and nerves, being usually the only evidence of its presence (fig. 108, Pl. 9). But along the upper surface of the nerve-cord the 'neurilemma' is generally a thick, loosely constructed layer of spongy cells (figs. 108, 109, Pl. 9). In the neuropilem the fibres are disposed chiefly longitudinally, though, contrary to the statement by Schmidt (1895), commissural fibres are also present (they are shown in Text-fig. 16 B). Running the length of the nerve-cord there is a kind of median septum in the neuropilem,

formed of irregularly constructed cells in which the nuclei usually appear rather larger and paler than those of the ganglionic tissue. It seems to be a form of neuroglial tissue. Scattered nuclei of similar appearance are found in numbers along the boundary of the neuropilem and the ganglion cells (fig. 109, Pl. 9).

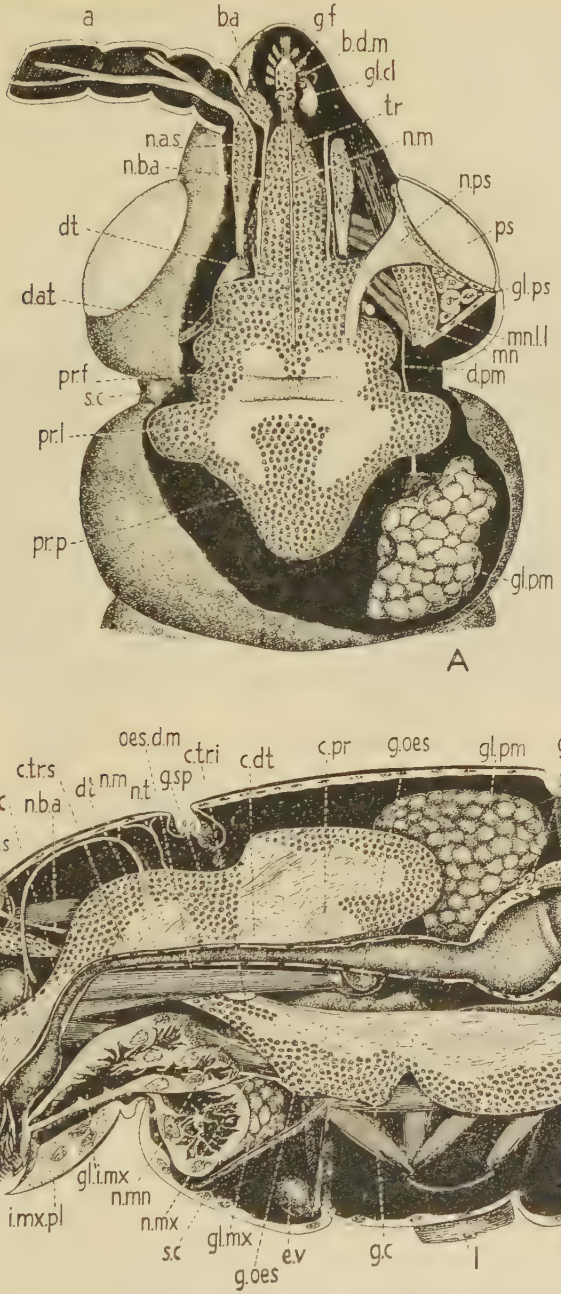
(ii) *The Brain* (Text-figs. 16, 17). This is comparatively large, its hinder end intruding far into the second abdominal segment, where it often impinges on the anterior end of the mid-gut. Seen from above it is roughly triangular, with the apex of the triangle directed forward. The protocerebrum is relatively large, and forms the hinder part of the brain. It is itself composed of three separate lobes, of which the posterior (pars intercerebralis) protrudes backwards and presents scarcely any indication of its originally paired condition. This posterior lobe forms a kind of inverted trough which arches over the oesophagus, and has its lateral margins almost in contact with the ventral nerve-cord. Anterior to it are the lateral and frontal lobes, the former widely expanded, but the latter bending downward and therefore only partially visible from above. Much of the superior and inferior surface of the protocerebrum is free from nerve-cells, the latter being distributed as a thick cortex mainly on its lateral and posterior walls. From the latter, however, the cortex encroaches from some distance on its inferior surface, and there is also a conspicuous median aggregation of nerve-cells on the dorsal surface of the brain.

The deutocerebrum, which is narrower than the protocerebrum, lies anterior to the latter; it has two lateral expansions, which extend in the direction of the pseudoculi, but otherwise presents little evidence of its originally paired condition. In a groove between the deutocerebrum and the frontal lobes of the protocerebrum lies, on each side, the ascending arm of the hypopharyngeal hypophysis.

Immediately below the deutocerebrum, and even intruding a little into it, is a median septum of ganglion cells that extends down from the roof of the brain at the junction of protocerebrum and deutocerebrum. It is indicated by *g.sp* in Text-figs. 16, 17, 18 A, C. As Text-fig. 18 C shows, it is actually a paired septum, for there is a thin partition of non-ganglionic cells between its two halves. Its development shows that it is not part of the deutocerebrum at all, but that it arises from a separate ganglion-Anlage, which is itself quite distinct from the protocerebrum. The possibility that it is the vestige of the pre-antennary ganglion, i.e. ganglion of the first head-segment, is indicated; but as the evidence for this cannot be conclusive, I shall speak of it as the 'septal ganglion'.

The tritocerebral ganglion lies to the side of the oesophagus, and merges below into the sub-oesophageal ganglion (Text-fig. 17); there are, therefore, no free circumoesophageal connectives. At their upper ends the two tritocerebral ganglia unite with one another, above the oesophagus, to form the most anterior part of the brain.

The following cerebral nerves can be distinguished (Text-figs. 16, 17):
(i) A pair of relatively large nerves from the pseudoculi (*n.ps*). These curve



TEXT-FIG. 16. Anterior end of adult, drawn to show principally the nervous system and cephalic glands.

A. View from above. B. Bisected animal.

Lettering. *a* antenna; *b.a* basal antennal sense organ; *b.d.m* buccal dilator muscles; *c.dt* deutocerebral commissure; *c.pr* protocerebral commissure; *c.tr.i* inferior tritocerebral

back along the hinder margin of the lateral expansions of the deutocerebrum, and enter the frontal lobes of the protocerebrum.

(ii) A single very long and thin unpaired 'median nerve' (*n.m*), that originates from the roof of the deutocerebrum, and later divides into right and left branches that end in the frontal ganglion of the stomatogastric system. This nerve may perhaps be equivalent of a nerve described by Fahlander (1938) from the chilopod brain; here it arises 'at the boundary between proto- and deutocerebrum' by a pair of roots, and passes to the frontal ganglion.

(iii) A pair of thin 'tegumentary nerves', that arise from the septal ganglion, just behind the base of the 'median nerve'. It is probable that they are sensory nerves associated with the setae on the roof of the head.

(iv) A pair of large antennary nerves (*n.a.s*) that arise from the deutocerebrum just below the recurving nerves from the pseudoculi, and are swollen with a thick investment of nerve-cells before entering the antennae. They do not seem to supply the muscles of the antennae, and are chiefly, if not exclusively, sensory.

(v) A pair of presumably motor nerves (*n.a.m*), that arise from the deutocerebrum just below the foregoing. They give off several branches to the muscles at the bases of the antennae, and then enter the latter, probably to supply its muscles.

(vi) A pair of thin nerves (*n.b.a*) arising from the deutocerebrum above the antennary nerves, and supplying the basal antennary sense organ (see section 14 (iv)).

(vii) A pair of relatively large clypeal nerves (*n.cl*), arising from the sides of the tritocerebrum at the tip of the brain. They pass along the floor of the clypeus, but I have not been able to observe their termination. They are evidently the equivalent of the labral nerves of other myriapods and of insects.

(viii) A short median unpaired connective from the tritocerebrum to the frontal ganglion of the stomatogastric system. It is lightly clothed with nerve-cells, and is remarkable for the fact that it lies, as an unpaired connective, above the oesophagus.

I have been able to make only scanty and inadequate observations on the internal structure of the brain. In the protocerebrum there is no recognizable

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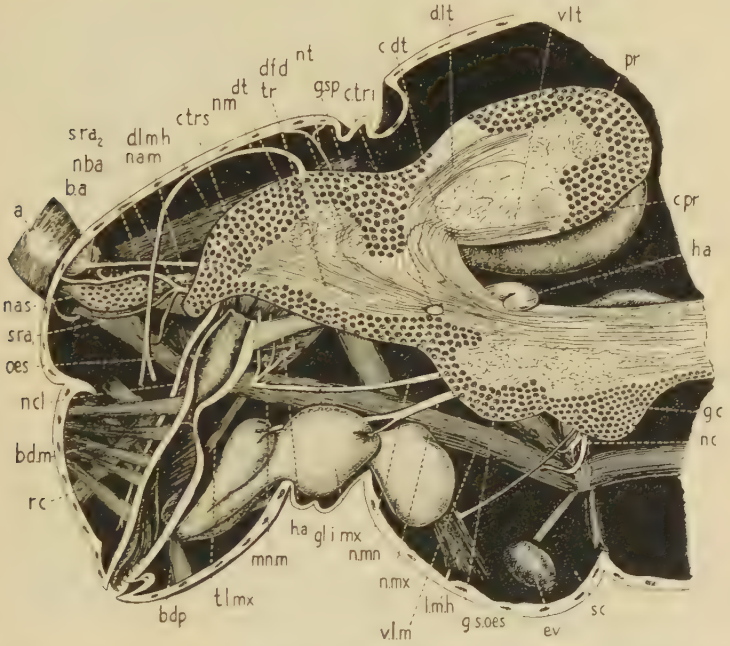
commissure; *c.tr.s* superior tritocerebral commissure; *d.a.t* dorsal arm of tentorium; *d.pm* duct of premandibular gland; *dt* deutocerebrum; *e.v* exsertile vesicle?; *f.c* connective between tritocerebrum and frontal ganglion; *g.c* ganglion of collum segment; *g.f* frontal ganglion; *g.oes* oesophageal ganglion; *g.s* stomachic ganglion; *g.s.oes* sub-oesophageal ganglion; *g.sp* 'septal ganglion'; *gl.cl* clypeal gland; *gl.i.mx* intermaxillary gland; *gl.mx* maxillary gland; *gl.pm* pre-mandibular gland; *gl.ps* pseudocular gland; *i.mx.pl* intermaxillary plate; *l* first leg; *mn* mandible; *mn.l.l* lateral ligamentous connexion between mandible and head-capsule; *n.a.s* sensory nerve to antenna; *n.b.a* nerve to basal antennal sense organ; *n.m* median nerve from deutocerebrum to frontal ganglion; *n.mn* mandibular nerve; *n.mx* maxillary nerve; *n.ps* pseudocular nerve; *n.t* tegumentary nerve; *oes.d.m* oesophageal dilator muscle; *pr.f* frontal lobe of protocerebrum; *pr.l* lateral lobe of protocerebrum; *pr.p* posterior lobe of protocerebrum; *ps* pseudoculus; *s.c* collum segment; *tr* tritocerebrum.

differentiation of globuli cells, in which respect the brain of *Pauropus* recalls that of Symphyla (Holmgren, 1916) and of *Geophilus* (Fahlander, 1938). Traversing the neuropilem are three pairs of compact fibrous tracts, which are



TEXT-FIG. 17. Bisected anterior end of adult *Pauropus*, drawn to show brain, sub-oesophageal ganglion, and collum ganglion, together with the nerves that arise from them. The frontal visceral ganglion has been omitted in order to display the clypeal nerve; the main mass of the tritocerebrum has been shown by omitting the oesophagus and oesophageal dilator muscles (shown in Text-fig. 16 B).

Lettering. As in Text-fig. 16.

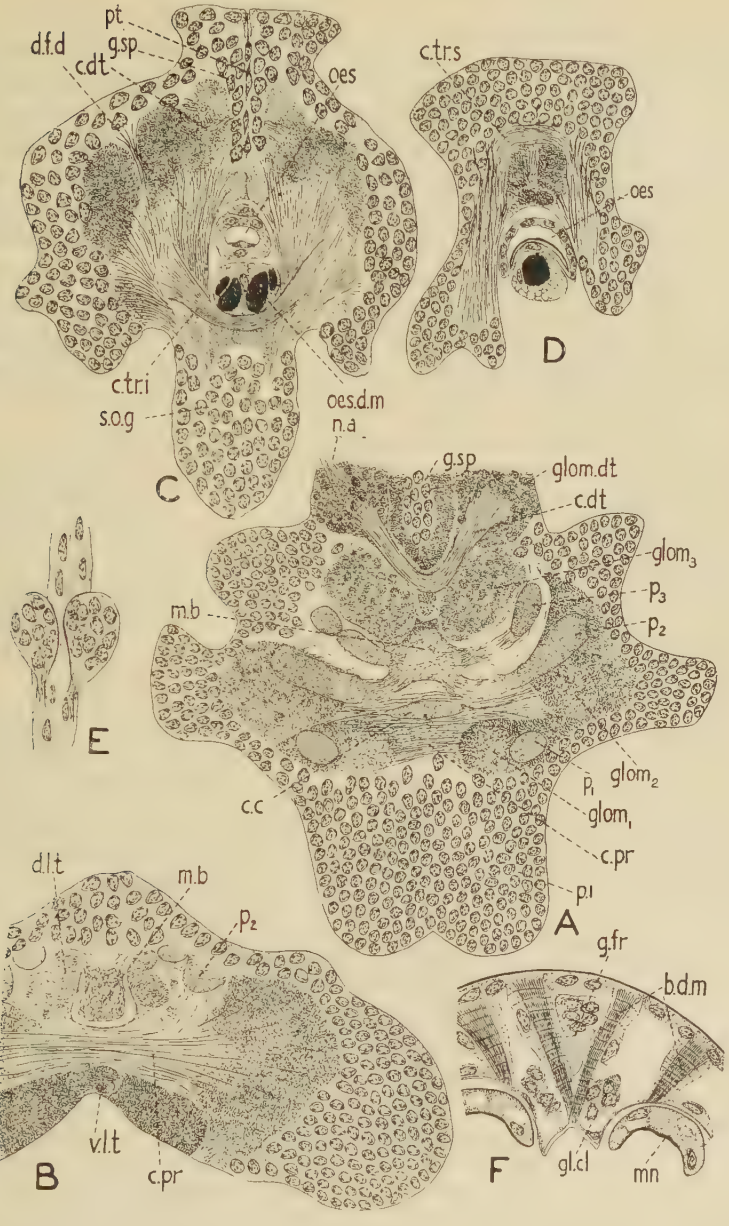
Additional lettering. *b.dp* buccal depressor muscle; *d.f.d* descending fibres of deutocerebrum; *d.l.m.h* dorsal longitudinal muscle of head; *d.l.t* dorsal longitudinal tract of descending fibres; *h.a* hypopharyngeal apophysis; *l.m.h* levator muscle of head; *mn.m* musculature of mandible; *n.a.m* antennary (motor) nerve; *n.c* nerves from collum ganglion; *n.cl* clypeal nerve; *n.t* tegumentary nerve; *oes* oesophagus; *pr* protocerebrum; *r.c* retractor muscle of clypeus; *s.r.a*$_{1,\ 2}$ sternal rotator muscles of antenna; *t.l.mx* tergal levator muscle of maxilla; *tr* tritocerebrum; *v.l.m* ventral longitudinal muscle; *v.l.t* ventral longitudinal tract of descending fibres.

evidently the stalks of the pedunculate bodies (p_1, p_2, p_3, Text-fig. 18 A), but in the absence of globuli cells their precise limits of origin within the cortex cannot be determined. The second and third peduncles, and probably also the first, are associated with a median mass of neuropilem, which is evidently the equivalent of the 'medial body' (Medialkörper) described by Holmgren for the brain of *Julus* and some chilopods. Three conspicuous paired masses of neuropilem, showing faint indications of glomerular structure, are present (*glom*$_{1,\ 2,\ 3}$), and these are perhaps the glomeruli associated with the peduncles. Immediately to the rear of the medial body is a small, transversely elongate

body of neuropilem, which has connexions with the lateral, and perhaps also frontal, lobes of the protocerebrum. It is evidently the corpus centrale. There are no ganglion cells associated with it, in which respect it resembles the corpus centrale of other myriapods and of most crustacea, but not of arachnids. Two commissures are distinguishable within the protocerebrum, viz.: (i) the principal commissure (Text-fig. 18 A, B), lying partly behind and partly below the medial body, and connecting the lateral lobes of the protocerebrum, and perhaps also deriving fibres from the pars intercerebralis, and (ii) a small commissure, which passes between the anterior glomerular masses, and apparently connects the two frontal lobes of the protocerebrum. Finally, reference must be made to three other fibre-tracts, (i) a pair of tracts connecting the neuropilem of the deutocerebrum with the lateral mass of glomerular neuropilem; (ii) a tract of fibres (*d.l.t*, Text-figs. 17, 18 B), connected with cells of the pars intercerebralis, and passing forward along the roof of the brain to enter the sub-oesophageal ganglion by way of the tritocerebrum; (iii) a tract of fibres (*v.l.t*, Text-figs. 17, 18 B), also connected with the pars intercerebralis, but passing along the floor of the brain into the deutocerebral commissure, beyond which they cannot be followed; some fibres of this tract seem to end in the neuropilem of the deutocerebrum itself. Many of the fibres considered under (2) and (3) are efferent fibres, for they can be seen to arise from cells in the pars intercerebralis. Similar fibres are shown by Holmgren in the brain of *Julus*.

The neuropilem of the deutocerebrum shows only a faint indication of glomerular structure; within this neuropilem most of the fibres of the sensory nerves from the antennae end (Text-fig. 18 A). The motor nerves to the antennae seem to be derived mainly from cells in the lateral wall of the deutocerebrum. From the roof of the deutocerebrum tracts of fibres bend down, near the midline, and, lying in front of those from the protocerebrum, pass by way of the tritocerebrum into the sub-oesophageal ganglion (*d.f.d*, Text-figs. 17, 18 A, C). The connexion of the deutocerebrum with the lateral glomerular mass of the protocerebrum has already been referred to; also the probable connexion with the pars intercerebralis. The deutocerebral commissure is very conspicuous, its component fibres curving backwards round the septal ganglion (Text-fig. 18 A).

A large part of the tritocerebral ganglion is made up of tracts of fibres that pass between the three component ganglia of the brain and the sub-oesophageal ganglion (Text-fig. 17). The clypeal nerves originate from ganglion cells in the wall of the tritocerebrum, a little anterior to the bases of the antennary nerves, i.e. in a 'pre-oral' position. Nerve-fibres from the lateral wall of the tritocerebrum can be seen passing upward into the main mass of the brain, but cannot be followed individually. The most noteworthy feature of the tritocerebrum is the presence within it of two commissures, a superior and an inferior. The latter (*c.tr.i*, Text-figs. 17, 18 C) is, as usual, sub-oesophageal, but it is not completely 'free' as in other myriapods and insects. The presence of a superior ('pre-oral') tritocerebral commissure is unexpected. Its fibres can be traced some distance down the tritocerebral ganglion, and some, at



Text-fig. 18. Histology of Brain and Stomatogastric Ganglia.

A. Horizontal section through protocerebrum and portion of deutocerebrum. B. Transverse section through protocerebrum, to show principal protocerebral commissure. C. Transverse section through brain to show inferior tritocerebral commissure. The section passes (above) through the deutocerebrum and 'septal ganglion' and (below) through the anterior end of the sub-oesophageal ganglion (for orientation cf. Text-fig. 17). D. Section through anterior tip of brain, showing superior tritocerebral commissure; at the sides of the oesophagus the section grazes along the anterior wall of the tritocerebrum. E. Section

least, of the crossing fibres can be seen to originate from ganglion cells in the roof of the tritocerebrum itself (Text-fig. 18 D). There seems to be no doubt as to the identification of this pre-oral tip of the brain as tritocerebrum, for the clypeal nerves and frontal connective both arise from it. The presence of a 'pre-oral' tritocerebral commissure in *Pauropus* supports the contention of Heymons (1901) that the position of the cerebral commissures in relation to the stomodaeum is determined, not by the site of origin of the ganglion in relation to the stomodaeum, but by the position into which they have moved at the time the commissures begin to develop. It may be recalled that St. Remy (1887) reported a pre-oral tritocerebral commissure in certain chilopods, though the recent work of Fahlander (1938) does not seem to support it.

(iii) *The Visceral Nervous System.* No description of this system in *Pauropus* has hitherto been given; its examination, indeed, presents considerable difficulty, and I can give only a general outline of its topography.

A stomatogastric and 'caudal' system are both present. The former seems to consist of four ganglia, as follow:

(i) A single frontal ganglion (*g.f*, Text-fig. 16 A, B), lying on the floor of the clypeus, wedged in between the inner group of buccal dilator muscles (Text-fig. 18 F). It is joined to the anterior tip of the brain (tritocerebrum) by the single short median connective above referred to. From the ganglion short nerves pass forward to the walls and floor of the clypeus. I have not been able to detect a recurrent nerve.

(ii) A pair of small oesophageal ganglia (*g.oes*, Text-fig. 16 B) that lie close together under the oesophagus, behind the posterior tips of the hypopharyngeal apophyses. They are connected by loose membrane with the immediately overlying protocerebrum and with the underlying mandibular ganglion; I cannot with certainty recognize any nerve-connexions between the oesophageal ganglia and either of these two ganglia, but it is clear that there is a connexion with nerve-fibres that can be followed a short distance along the wall of the oesophagus. In view of their remarkable development described below (they develop from cells that migrate in from the ends of the ingrown mandibles), comparison with the corpora allata of insects is at once suggested; in their microscopic structure, however, they show no evidence whatever of glandular structure, but on the contrary resemble ganglionic tissue (Text-fig. 18 E).

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along inferior wall of oesophagus, showing the oesophageal ganglia; anterior end directed downward. F. Transverse section through clypeus, to show frontal ganglion. Both groups of buccal dilator muscles are seen in the section, and between them the clypeal gland.

Lettering. *b.d.m* buccal dilator muscles; *c.c* corpus centrale; *c.dt* deutocerebral commissure; *c.pr* principal commissure of protocerebrum; *c.tr.i* inferior commissure of tritocerebrum; *c.tr.s* superior commissure of tritocerebrum; *d.f.d* descending fibres of deutocerebrum; *d.l.t* dorsal longitudinal fibre tracts; *dt* deutocerebrum; *g.fr* frontal ganglion; *gl.cl* clypeal gland; $glom_{1, 2, 3}$ first, second, and third masses of glomerular neuropilem; *glom.dt* glomerular neuropilem of deutocerebrum; *g.sp* septal (pre-antennary ?) ganglion; *m.b* medial body; *mn* mandible; *n.a* sensory nerve from antenna; *oes* oesophagus; *oes.d.m* oesophageal dilator muscle; $p_{1, 2, 3}$ first, second, and third peduncles; *p.i* pars intercerebralis; *pt* partition of non-ganglionic cells in the 'septal ganglion'; *s.o.g* sub-oesophageal ganglion; *v.l.t* ventral longitudinal fibre tract.

Tentatively, therefore, we may regard them as ganglia of the stomatogastric system, despite their peculiar manner of development.

(iii) A minute stomachic ganglion (*g.s*, Text-figs. 6 D, 16 B) that lies at the posterior end of the oesophagus, at its entrance into the mid-gut.

There is no ganglion corresponding to the hypocerebral ganglion of insects. I have not been able to detect interganglionic connexions on the oesophageal wall.

The 'caudal' system of visceral nerves (Text-fig. 15) arises from the terminal ganglion of the nerve-cord. In the adult animal a relatively large clump of nerve-cells is to be seen forming the terminal (visceral) lobe of the last abdominal ganglion. From this visceral ganglion a band of nerve-fibres, sparsely clothed with nerve-cells, passes back along the floor of the terminal segment on to the inferior surface of the rectum; no other nerves arise from it. The distribution of these fibres on the intestinal wall cannot be followed.

(*b*) *Development of the Nervous System*

(i) *The Ventral Nerve-cord.* In embryos aged 6 days, and at a time when the protocerebral ganglia are already in course of formation, sections through the germ-band still reveal no sign of the development of any of the ganglia of the ventral nerve-cord. This lag in the formation of the ventral nerve-ganglia is readily seen in fig. 58 A, B, Pl. 5; the two sections are from the same embryo, fig. A showing, in the lower half of the section, the initial thickening of the head-wall, while in no other part of the section is there even an indication of the development of ganglion-Anlagen.

In rather older embryos, in which somites are in process of forming, the ectoderm along the entire post-oral length of the germ-band has become gathered into a pair of thickenings, between which only a very narrow strip of median ectoderm intervenes. This may be seen in figs. 63, 65, Pl. 5; fig. 74, Pl. 7, the three sections being taken respectively through the maxillary, collum, and second abdominal segments of a single embryo. Within the thickened bands of ectoderm the crowded nuclei lie, in places, several deep, but do not yet present any orderliness of arrangement. Between the bands of ectoderm is the unsegmented median mesoderm.

During the seventh day the developing somites begin to move into a more lateral position along the widening germ-band, and therewith the first indications of the ganglion-Anlagen become apparent (fig. 64, Pl. 5; fig. 75, Pl. 7). The latter lie to the sides of the median mesoderm and medial to the row of somites, and are directly exposed to the yolk. Lateral to them, and covered above by the somites, the ectoderm is thinner, and will become the epidermis of the appendages.[1]

In sections through rather more advanced embryos, the germ-band appears considerably thickened, and now, for the first time, the nuclei begin to display

[1] In examining the many drawings of sections through the germ-band the reader will be struck by the apparent absence of appendage-rudiments below the somites. In whole embryos the appendages are easily seen; but in section they are scarcely recognizable because they are, at first, only gentle elevations of the epidermis, and the furrow which delimits them is, in section, hard to distinguish from natural clefts between the cells.

an orderliness of arrangement, with their long axes directed towards the surface (fig. 67 B, Pl. 6; upper half of section). Within the developing ganglia the nuclei near the surface begin to recede a little, and therewith the 'ventral organs' begin to form (fig. 76, Pl. 7); the cells immediately above them constitute the ganglion-Anlagen proper. The 'ventral organs', of which there is a single pair in each segment except the last abdominal, develop first in the more anterior segments, whence their formation spreads progressively into the segments behind. The ganglion-Anlagen of successive segments do not yet form an unbroken chain, being partially interrupted by narrow clefts at the intersegments (fig. 105, Pl. 9; Text-fig. 7).

During the ninth day the ganglia begin to take shape. They now form bulging masses on the floor of the germ-band, the clefts between successive ganglia gradually disappearing (fig. 103, Pl. 9); but the ganglion-halves still remain apart, with the median mesoderm intervening as a wedge from above. Mitoses appear in great abundance, in the cells both of the 'ventral organs' and of the ganglion-Anlagen (fig. 66, Pl. 5; figs. 86, 88, Pl. 7). The 'ventral organs' have now, also, become much more distinct. They are shown in fig. 66, Pl. 5; figs. 86, 88, Pl. 7; fig. 103, Pl. 9; Text-fig. 7.

These 'ventral organs' are, indeed, very peculiar cell-formations. Their cells are long and spindly, and tend to radiate inwards from a point on the surface. Often they display a gentle surface depression (cf. fig. 103, Pl. 9). The nuclei become withdrawn to the inner tips of the cells, in consequence of which the 'organs' become very conspicuous in sections as pale fan-shaped structures that contrast strongly with the adjacent deeply staining ganglion tissue. In order that the reader may form a better judgment of them, I have included two photographs (fig. 104 A, B, Pl. 9) of parasagittal sections along a 9-day embryo, in which four of the 'ventral organs', those of the first three abdominal segments and of the maxillary segment, can be seen.

On the ninth or tenth day the neuropilem begins to develop, and therewith the ganglion-halves become welded into a single mass. This neuropilem appears first in the more anterior segments, from where its development spreads progressively backwards. Sections through ganglia at this stage of development are shown in figs. 78, 89, Pl. 7, the former being from the second abdominal segment of a 9-day embryo, the latter from the collum segment of a 10-day embryo. The neuropilem arises as a pair of diminutive masses in the upper half of each ganglion-half, and is not roofed in by the ganglion-cells. Spreading medially, it covers in the median mesoderm, which thereby becomes included within the ganglion itself. Out of this median mesoderm will develop the median septum of neuroglia tissue, to which reference has already been made above. The ganglia are, at this stage, still associated with their 'ventral organs', and both in these and in the ganglia themselves mitosis is active. With further enlargement of the ganglia, and accumulation of neuropilem, the cleft separating the ganglion-halves becomes more and more reduced (fig. 98, Pl. 8), till eventually it vanishes (fig. 95, Pl. 8). The inclusion of the median mesoderm within the ganglia in *Pauropus* seems to be unique; in

Scolopendra also a median septum of neuroglia tissue is present, but in this case it apparently arises from the median cord (Mittelstrang) ectoderm (Heymons, 1901).

The fate of the 'ventral organs' is not identical in all the segments. In the ganglia of the three leg-bearing segments these structures become incorporated into the ganglia themselves. Up to the ninth or tenth day a narrow median strip of ectoderm, not more than two or three cells in width, has intervened between the two rows of 'ventral organs' (fig. 78, Pl. 7). These cells now begin to spread out, covering in the 'ventral organs' from below. The latter, already considerably reduced in size, thereby become shut off from the epidermis, and are now part of the ganglia (fig. 106, Pl. 9). Their cell-orientation is soon lost, and thereafter they cannot be distinguished from the ganglion tissue.

The 'ventral organs' of the collum segment are only partially absorbed into the ganglia, their vestiges remaining in the surface ectoderm, to become converted later into the 'exsertile vesicles' of that segment (section 14 (v)). The 'ventral organs' of the maxillary and mandibular segments do not become part of the ganglia. In the maxillary segment they remain in association with the Anlage of the intermaxillary gland, and eventually become partly absorbed into that gland, and partly into the adjacent epidermis (see section 10 (iv)). Those of the mandibular segment come to occupy part of the floor of the pre-oral cavity between the mandibles (fig. 70, Pl. 6; fig. 94 D, Pl. 8); they also become separated from the ganglia, being recognizable for a short time as a thickening on the floor of the pre-oral cavity, but their cell-orientation is soon lost. They do not seem to give origin to any recognizable structure in the larva. In Symphyla the superlinguae are formed from them, but I cannot obtain certain evidence for this in *Pauropus*.

It should be observed that in *Pauropus* there is no incorporation of 'median cord' (Mittelstrang) ectoderm into the ganglia, as in Symphyla, Chilopoda, and Insecta.

Throughout the late embryonic and pupal periods both the neuropilem and the ganglionic tissue increase in quantity (figs. 107, 108, Pl. 9), and both longitudinal and commissural bundles of fibres soon become visible. Thereafter the successive ganglia become more clearly distinguishable (Text-figs. 8, 9). The sub-oesophageal ganglion forms in the late embryo by fusion of maxillary and mandibular ganglia. The collum ganglion, though less clearly demarcated than the succeeding ganglia, does not fully merge with the sub-oesophageal ganglion. An early stage in the formation of the latter ganglion is shown in fig. 99, Pl. 8.

The neurilemma seems to be derived directly from the cells of the ganglia; I have not found any evidence that the median mesoderm plays any part in its formation, though this might have been expected. Two such neurilemmal cells are shown in fig. 108, Pl. 9, where they are to be seen lying flattened out against the ganglia. The spongy neurilemmal cells on the roof of the neuropilem undoubtedly develop directly from cells of the nerve-cord. These cells may be seen in any of the following figures: figs. 95, 97, Pl. 8; figs. 107, 108,

109, Pl. 9. When first recognizable they are hardly to be distinguished from ganglion-cells (fig. 98, Pl. 8; fig. 106, Pl. 9).

Special consideration must be given to the development of the terminal ganglia, i.e. the ganglia posterior to the fourth abdominal ganglion. The development of these ganglia, like the mesoderm of these segments, is much delayed (cf. Text-fig. 7), there being no indication of them until about the ninth day. A 'ventral organ' cell-disposition then becomes apparent, and thereafter the ganglion-Anlage grows rapidly in size. A few days before the formation of the pupa, neuropilem appears in the more anterior part of this ganglionic tissue (Text-fig. 8). At the same time a constriction appears, involving both the ganglion and the 'ventral organ', which becomes separated into a more anterior part, containing the neuropilem, and a more posterior part in which neuropilem is not yet present. The more anterior of these is the developing fifth abdominal ganglion (fig. 86, Pl. 7). The hinder, however, is not, as might have been expected, the future sixth ganglion, but the locus from which the new ganglia successively develop in the growing larva. I shall speak of it as the 'teloblastic ganglion'. Its further development is described below (Post-emb. Dev., section 2).

Since there is a small somite in the anal segment, the formation of an anal ganglion is to be expected. The evidence for this is not, however, satisfactory. It seems that, in the advanced embryo, a few cells from the anal ectoderm do, in fact, become incorporated into the hinder end of the pre-anal ganglion (Text-fig. 8); but I have never seen any indication of a clearly defined anal ganglion, and there is certainly no 'ventral organ' in this terminal segment.

The most noteworthy feature of the developing nerve-cord of *Pauropus* is the presence of 'ventral organs' reminiscent of those of *Peripatus* and Symphyla. What is the significance of these peculiar structures? Kennel (1886) first described them in *Peripatus*, believing them to be the remains of a once-functional series of organs, of which a trace could still be seen in the adults of some species of *Peripatus*. The fact that, in Symphyla, their remains become converted into the exsertile vesicles seemed to support Kennel's view; and in *Pauropus* also the peculiar organs of the collum segment, themselves reminiscent of exsertile vesicles, also arise from 'ventral organs' (cf. section 10 (v)). Moreover, in the remarkable *Peripatus*-like *Xenusion*, of supposed pre-Cambrian age, each segment bore a pair of conspicuous protuberances, in the position of the 'ventral organs' (Heymons, 1928). In the embryo of *Scolopendra* the ganglia arise by the immigration of cells from invaginated pits, which themselves become incorporated into the ganglia. Heymons (1901), who described this, was led to compare them to Kennel's 'ventral organs'; but, in opposition to Kennel, he concluded that the 'ventral organs', far from being the vestiges of ancestral organs, were merely loci of cell ingrowth. But neither Kennel nor Sedgwick (1887) make any statement as to the derivation of ganglion-cells from the 'ventral organs'. In the embryo of *Hanseniella* also the ganglion-cells are mainly derived from a zone of unorientated cells between the 'ventral organs' and the ganglia, mitoses within the 'ventral organs'

being infrequent (Tiegs, 1940). In *Pauropus* mitoses are often met with in the cells of the 'ventral organs', but are in even greater abundance within the ganglia themselves; yet their eventual incorporation into some ganglia can leave no doubt that they are themselves a source of ganglion-cell formation. While this is, perhaps, not a crucial objection to Kennel's view, since it may be correlated with a reduction of exsertile vesicles in *Pauropus*, a more serious difficulty has arisen in the present work; for apparently normal 'ventral organs' appear in association with the frontal and lateral lobes of the protocerebral ganglia (see next section). These ganglia do not form part of the ventral series at all, but belong to the supra-oesophageal ganglion. Their presence in these ganglia suggests, therefore, that 'ventral organ' formation is, in some undisclosed way, bound up with the process of ganglion formation.

(ii) *The Brain*. This develops out of the following embryonic rudiments:

(i) The great protocerebral ganglia, themselves constituted out of three separate pairs of lobes, viz. the posterior, lateral, and frontal. These comprise the true supra-oesophageal ganglion, or ganglion of the acron (prostomium).

(ii) A pair of diminutive ganglia, probably to be regarded as the pre-antennary ganglia, or ganglia of the reduced first segment.

(iii) The antennary ganglia, or ganglia of the second segment, out of which the deutocerebrum will develop.

(iv) The pre-mandibular ganglia, or ganglia of the third segment, out of which will form the tritocerebrum.

There is no median unpaired ganglion corresponding to the 'archicerebrum' of Heymons. (The use of this term by Heymons has introduced some confusion into the subject. In Lankester's original form the name is applied to the ganglion of the annelid prostomium. Heymons correctly regards the entire protocerebrum as the equivalent of this ganglion, calling it, however, the 'syncerebrum', and reserving the term 'archicerebrum' for a small median unpaired component found in *Scolopendra* and *Forficula*. In Lankester's definition, however, the 'syncerebrum' connotes the ganglion of the acron, fused with a number of originally post-oral ventral ganglia.)

Almost from their first appearance the head-lobes of the embryo show a pair of pronounced lateral thickenings, the cells of which have a tendency to radiate inwards from the surface, after the manner of 'ventral organs' (fig. 58 A, Pl. 5). These thickenings are the rudiments of the posterior lobes of the protocerebral ganglia, and give the first indication of the nervous system. During the seventh day, the head-lobes having considerably enlarged, these first recognizable rudiments of the brain come to lie nearer together, on the roof of the head, from where they now begin to invaginate below the surface. Throughout the seventh day their slit-like orifices are conspicuous on the roof of the head (figs. 25, 26, Pl. 2), but during the eighth day they become closed.

Meanwhile the ectoderm of the enlarging head-lobes has markedly thickened, the zone of thickening spreading down almost to the developing pre-oral cavity. Within this thickened ectoderm a pair of new ganglionic masses may now be seen in course of formation, being located on the sides of the head,

between the invaginating posterior lobes and the stomodaeum. They are the indistinctly demarcated rudiments of the lateral and frontal lobes of the protocerebrum. These ganglion-rudiments are shown in fig. 68, Pl. 6, the section passing 'horizontally' along the head, ventral to the invaginated posterior lobes, no part of which is present in the section; cell-proliferation is in active progress, mitoses occurring mainly among the surface cells, but there is not yet evident among these any obvious orientation.

Confluent with the frontal lobes, and lying immediately to the side of the pre-oral mesoderm (future pre-antennary somites) are already to be seen the Anlagen of a pair of small ganglia. They lie just in front of the site of impending formation of the antennary ganglia, and give rise to a part of the brain quite distinct from the protocerebrum. I shall speak of them as the pre-antennary ganglia, implying a probable homology with the pre-antennary ganglia of *Scolopendra* embryos (Heymons, 1901). They soon become quite conspicuous, and are a constant feature of all embryos after about the eighth day. They give rise, moreover, to a well-defined part of the brain, viz. the 'septal ganglion' above described. I am bound to say that, as evidence for a pre-antennary segment, they are less satisfactory than might have been expected from so primitive a myriapod as *Pauropus*, for owing to crowding together of the head-ganglia they merge closely with the protocerebral lobes. They lie, moreover, lateral to the somites, and not, as might have been expected, median to them. This is probably in consequence of the remarkable displacement of segments which attends the formation of the pre-oral cavity. It should be observed that it is not the pre-antennary ganglia but the somites which are in an unusual position, for these lie close together in front of the stomodaeum.

During the eighth day all the component ganglia of the future brain have become well defined. It is hardly possible to construct a single drawing which will depict them in relation to one another; from the series shown in figs. 67 A–F, Pl. 6, however, the reader will be able to visualize them. The series represents six successive sections cut 'horizontally' through the head of an 8-day embryo. In fig. A the section passes just under the posterior lobes of the protocerebral ganglia, which are growing down from the roof of the head, only a fragment of the ganglion on the right side being visible. In the lateral and frontal lobes the more superficially placed nuclei are beginning to recede, and therewith a typical 'ventral organ' cell-disposition arises. This is also seen in the pre-antennary ganglion, which has itself now become quite distinct. In figs. B and C the large antennary ganglia are seen, lying immediately behind (below) the pre-antennary ganglia; the left ganglion already shows distinct 'ventral organ' structure. The antennae have by this time moved into a position anterior to the pre-oral cavity, the ganglia lying just medial to their bases, and therefore, unlike the more posterior ganglia, widely separated from one another. The pre-mandibular ganglia are also developing, and display a 'ventral organ'. They lie at this period immediately to the rear of the developing pre-oral cavity, only subsequently becoming invaginated into it.

They are shown in fig. F. Their post-oral position is best seen in fig. 80, Pl. 7, which is from an embryo of about the same age.

During the eighth day the peculiar radiating cell-disposition becomes more pronounced in the protocerebral ganglia, involving much of the frontal and lateral lobes (fig. 93, Pl. 8). Mitoses are in abundance throughout the enlarging ganglia. The pre-antennary ganglia have become fairly clearly demarcated from the frontal lobes. It is during this period, also, that the enlargement of the pre-oral cavity, which is proceeding, begins to draw in the 'ventral organs' of the pre-mandibular ganglia, which now come to form part of the hinder and lateral walls of this cavity. An early phase of this migration of the pre-mandibular epidermis is seen in fig. 92, Pl. 8; the drawing should be compared with figs. 67 E, F, Pl. 6, in which the ganglion is still completely post-oral in position. The pre-mandibular ganglia thereby come to lie more to the side of the pre-oral cavity, the mandibular ganglion now lying close along the hinder margin of the latter (Text-fig. 7; compare, also, fig. 80, Pl. 7, and fig. 103, Pl. 9).

During the ninth and tenth days all three lobes of the protocerebrum undergo much enlargement. The posterior lobes begin to merge with the lateral lobes, and lose connexion with the epidermis on the roof of the head, whence they originated. Their invagination cavities are now quite obliterated. Flattening out considerably, they come into almost direct contact with one another on the mid-dorsal surface of the head.

It is shortly after this, while the lateral and frontal lobes are still part of the epidermis, that the neuropilem is first seen (fig. 115 A, Pl. 9), and with its appearance the component lobes of the brain become still more firmly welded together (fig. 94, Pl. 8). The first-formed neuropilem is a transverse band passing between the lateral lobes, on the under surface and in front of the posterior lobes, and a large proportion of its axons comprise the protocerebral commissures. Thereafter the lateral and frontal lobes of the protocerebrum begin to separate away from the epidermis. This is not attended by a 'bending in' of the ganglionic masses, as is the case with the posterior lobes; instead, the adjacent epidermis closes in under them, and, together with their 'ventral organs', they lose connexion with the surface.

It is quite impossible to depict the concluding stages in the formation of the brain except by means of sections. For the purpose, reference should be made to the series of 'horizontal' sections shown in figs. 94 A, B, C, Pl. 8, to the frontal sections shown in figs. 110, 111, Pl. 9, and to the oblique section depicted in fig. 101, Pl. 8. During the tenth day the deutocerebrum is seen in process of formation. The antennary ganglia have, by this time, lost connexion with the epidermis, their 'ventral organs' becoming absorbed into them. Though the main mass of the ganglia still lies under the frontal lobes to the side of the oesophagus (fig. 110, Pl. 9), a median fusion of opposite ganglia has taken place above the oesophagus, and within this the neuropilem is already appearing (fig. 94 B, Pl. 8; fig. 111, Pl. 9; Text-fig. 8). Under this, ventro-lateral to the oesophagus, are the pre-mandibular ganglia (developing tritocerebrum) (figs. 110, 111, Pl. 9; fig. 116 A, Pl. 10): in appropriately directed sections their

'ventral organs', which are now well within the pre-oral cavity, may be seen in process of separating from the latter, thereby to become incorporated into the ganglia; at the same time the inferior tritocerebral commissure is being formed below the oesophagus (fig. 94 C, Pl. 8; fig. 116 A, Pl. 10). The pre-mandibular ganglia are now in partial continuity with both antennary and mandibular ganglia; the neuropilem between them is developing (fig. 101, Pl. 8; fig. 116 A, Pl. 10).

Special interest attaches now to the pre-antennary ganglia. With the enlargement of the frontal lobes of the protocerebrum, these ganglia are carried into an almost median position (fig. 101, Pl. 8; compare this with fig. 67, Pl. 6), but are prevented from meeting by a septum of epidermal cells that grow down from the roof of the head. This septum is shown in Text-fig. 8 and in fig. 101, Pl. 8, fig. 111, Pl. 9, and fig. 117, Pl. 10. The pre-antennary ganglia, as fig. 111 shows particularly clearly, lie between the deutocerebrum and the frontal lobes of the protocerebrum; in Text-fig. 8 the position of the epidermal septum also clearly defines their position. As fig. 101, Pl. 8, and figs. 116 A, 117, Pl. 10, show, the pre-antennary ganglia lie median to the neuropilem. There can no longer be any doubt that they are the 'septal ganglia', above referred to, in course of development. The epidermal partition between them is detectable even in the adult animal (cf. Text-fig. 18 C).

With continued growth of the ganglionic tissue and neuropilem, the brain enlarges more and more, till in the pupa its hinder end has intruded into the abdomen, just into its third segment (Text-fig. 9). It comes thereby to assume an increasingly horizontal position, the deutocerebrum lying no longer below, but actually in front of the protocerebrum, with which it is now closely merged. The pre-mandibular ganglia, hitherto completely post-oral in position, have meanwhile also begun, at their upper ends, to grow over the oesophagus (fig. 99, Pl. 8), and thereby come to form the anterior tip of the brain. The greater part of their substance remains, however, to the side of the oesophagus, so that 'free' connectives with the sub-oesophageal ganglion do not develop. The superior tritocerebral commissure is already distinguishable before the pupa forms.

(iii) *The Visceral Nervous System.* The visceral ganglia appear much later than the rest of the nervous system.

The frontal ganglion of the stomatogastric system is first seen in advanced embryos in which the neuropilem is already forming in the rest of the nervous system. It arises as a single, small, and unpaired median thickening of the roof of the oesophagus, near the hind end of the buccal dilator muscles (Text-fig. 8; fig. 99, Pl. 8); immediately behind it, to the side of the oesophagus, are the pre-mandibular (tritocerebral) ganglia. The rudiment of the ganglion soon becomes almost completely detached from the stomodaeal wall, and may now be seen wedged in between the hinder fibres of the buccal dilator muscles (fig. 91, Pl. 7). Pressing against it from behind are the upper tips of the pre-mandibular ganglia, which have now fused with one another above the oesophagus. From the point of fusion of these ganglia a short median band of

neuropilem with longitudinal axons is soon seen joining the frontal ganglion, forming its connective with the brain. Even in early pupae, however, the ganglion itself seems to remain free from neuropilem, and the latter does not become recognizable until the time that the larva emerges.

The oesophageal ganglia develop in such a surprising manner, that some doubt must be entertained as to whether they are, in truth, ganglia. They arise from the inner ends of the mandibular apodemes. In describing their development from the latter, some observations relating to the formation of the apodemes and associated parts may be included. In the 9-day embryo the ectoderm around the lateral margin of the mandibles begins to grow, as an apodeme, into the head, drawing in with it the mesoderm that is clumped at the base of the appendage (cf. section 8 (iv)). In the 10-day embryo these apodemes intrude backward in the head, as far as the superior surface of the mandibular ganglion. The developing hypopharyngeal apophyses, which have by this time begun to form (see section 14 (vi)), lie between them and the ganglion (fig. 94 D, Pl. 8). During the tenth day cells begin to separate from the inner tips of the apodemes, and grow, under the hypopharyngeal apophyses, towards one another across the upper surface of the mandibular ganglion. Fig. 115 A, Pl. 9, shows the initial phase of this process. In the rather later embryo shown in fig. 116 B, Pl. 10, there has now been formed a continuous band of cells uniting the ends of the mandibular apodemes across the mandibular ganglion; it lies immediately to the rear of the tritocerebral commissure (cf. fig. 116 A, Pl. 10). On the left side, in fig. 116 B, the section extends along a considerable length of the mandible, and shows incidentally the development of the 'lateral ligament', which will later bind the tip of the mandible to the lateral head-wall; this ligament is shown in fig. 111, Pl. 9. In the still later embryo shown in fig. 117, Pl. 10, the band of cells uniting the mandibular apodemes is beginning to develop a pair of swellings, and therewith we see the first indication of the actual ganglia; incidentally, it may be noted, a new feature has appeared in this section, namely, a connexion between the apophysis and the mandibular apodeme, and this constitutes the 'median ligament' of the latter (see section 6 (ii) (*b*)). Finally, in the advanced embryo the connecting band between the apodemes disappears, its cells being wholly concentrated in the two oesophageal ganglia (figs. 118, 119, Pl. 10). (The reader who compares fig. 118 with fig. 117 will suspect that the oesophageal ganglia of fig. 118 are surely identical with the rounded bodies labelled 'tritocerebrum' in Fig. 117. It should be explained that the hindermost tips of the tritocerebral ganglia intrude a little into the section from in front (see especially fig. 116 A, B) but are quite distinct from the oesophageal ganglia. For the relation of the latter to the tritocerebrum, see fig. 119, Pl. 10.) The ganglia, at this period, show no obvious association with the oesophagus; but in the pupa we see them clearly attached to the under surface of the latter.

The remarkable manner of formation of the oesophageal ganglia, not from the oesophageal wall, but from the bases of the mandibles, recalls current descriptions of the formation of the corpora allata of insects rather than of the

oesophageal ganglia. In *Calandra* the corpora allata come from the antennary mesoderm (Tiegs and Murray, 1938); but in all other cases investigated—*Forficula*, *Gryllus* (Heymons, 1895); *Bacillus rosii* (Heymons, 1897); *Chalicodoma* (Carrière and Bürger, 1898); *Formica* (Strindberg, 1913); *Apis* (Nelson, 1915); *Pieris* (Eastham, 1930); *Carausius* (Wiesmann, 1926; Pflugfelder, 1937)—they develop out of the ectoderm between the bases of the mandibles and maxillae. There is, however, nothing in the structure of the oesophageal ganglia of *Pauropus* to suggest any affinity with corpora allata, for they present no indication of glandular structure. In the phasmid *Carausius*, where the development of the 'pharyngeal ganglia' has been specially investigated (Wiesmann, 1927; Pflugfelder, 1937), they are found to arise from the oesophageal wall in close association with the hypocerebral ganglion. Here they appear to be of mixed glandular and nervous nature (corpora cardiaca), and cannot therefore be compared with the oesophageal ganglia of *Pauropus*.

The small stomachic ganglion develops in the usual way, by separation of cells from the hind end of the fore-gut (Text-fig. 8).

The caudal system of visceral nerves is a part of the terminal abdominal ganglion, and its development does not, therefore, present any special problem. The visceral ganglion does not, indeed, become demarcated from the terminal abdominal ganglion until late in the fourth larval stadium.

14. *The Epidermis and Some Simple Derivatives*

(i) *The Epidermis.* The differentiation of the blastoderm, during the fifth day, into provisional body-wall and germ-band, involves a gradual thinning out of the former, with attendant flattening of cells; and, in the germ-band, an increase in thickness, its closely crowded cells becoming columnar, with no very clear indication of delimiting cell-walls. In later embryos the ectoderm of the germ-band becomes even thicker, the nuclei lying often several deep (figs. 63, 65, Pl. 5); in some preparations the delimiting of cells in the ectoderm and other parts now appears quite pronounced, while in other preparations it is not perceptible. In the 'ventral organs', with their tapering and radiating cells, cell-demarcations are particularly clear (fig. 104, Pl. 9).

The great thickening of the ectoderm of the germ-band is, of course, associated with the development of the chain of nerve-ganglia and of the appendages. When the ganglia separate away from the epidermis the latter becomes reduced to a comparatively thin membrane. The epidermis of the appendages also thins out when these elongate.

During the eighth or ninth day a process of epidermal thickening begins to spread from along the margin of the germ-band over the provisional body-wall. As it proceeds, intersegmental grooves make their first appearance along the lateral walls of the embryo, their cells having a distinctly fusiform character. In the advanced embryo (fig. 29 A, Pl. 3) the thickening has spread on to the mid-dorsal surface, where the intersegmental grooves are now exceptionally deep. On the pleural walls of the abdominal segments the epidermis remains unusually thin, with widely spaced nuclei, and through it the yolk

may still be seen. It should be observed that the cells of the provisional body-wall do not degenerate before the advancing zone of epidermal thickening, but are themselves involved in the thickening, for mitoses are abundant among them.

The embryonic and pupal cuticles, to which reference has already been made in section 6 (ii), develop on about the eighth and twelfth day respectively. The definitive cuticle of the first instar larva does not form until late in the pupal period.

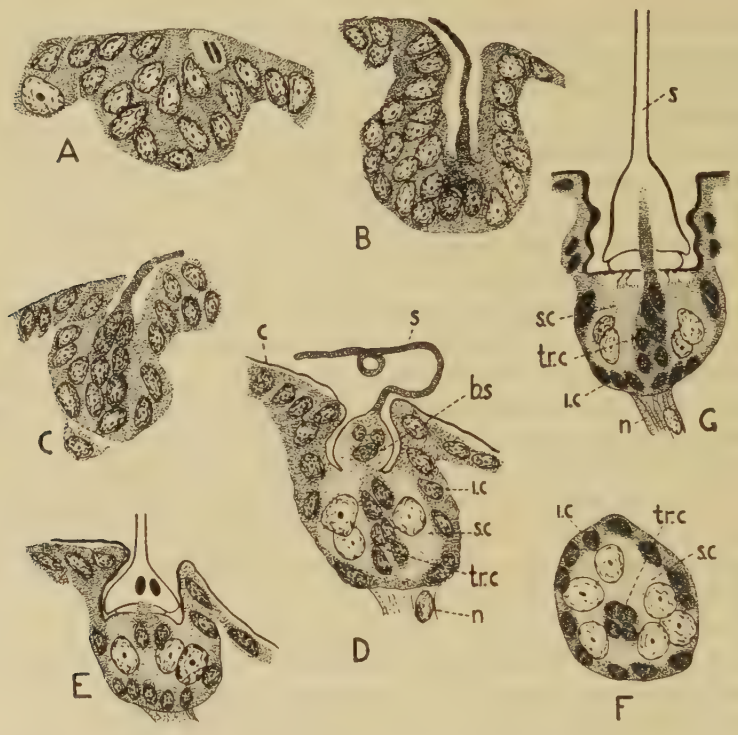
The great cutting setae of the first embryonic cuticle develop from large cells that appear in the epidermis during the ninth day (fig. 111, Pl. 9). These setigerous cells may still be seen in early pupae, but thereafter seem to become incorporated as ordinary cells into the epidermis, for I have not seen any in process of disruption.

The setae which adorn the body of *Pauropus* are remarkable for their variety of form. Most of them do not appear until a day before the larva emerges from the pupal sheath. The smallest setae are certainly the products of single epidermal cells, which grow beyond the surface of the epidermis and then chitinize. The extraordinary 'flagella' of the antennae, on the other hand, seem to arise by the co-operation of several cells. They begin to form in very young pupae (fig. 32 B, Pl. 3), two or three cells at the tips of the antennal rami sending out a common protoplasmic filament which thickens and then elongates, and assumes the peculiar annulated form of the flagellum. The flagella are hollow, and have a protoplasmic axis.

(ii) *The Trichobothria.* These are the great sensory setae, there being a single pair on each of the tergal scutes except the first and last (diminutive anal)—see Text-figs. 4, 23, 24. Most writers regard them as tactile sensilla; Verhoeff (1934), however, believes that as tactile organs they would be superfluous as the animal is already generously supplied with these, and suggests that they may be used for the detection of air currents. I find, however, that when a trichobothrium is gently touched with a fine needle the animal responds by turning swiftly to avoid the contact.

Each trichobothrium is a long, slender, chitinous seta, covered, for its greater length, with a very fine pubescence. At its base it has a bell-shaped expansion, which is sunk a little below the adjacent chitin. The underlying epidermis forms a thick bulbous swelling, from which the nerve passes down beside the dorso-ventral muscle into the hinder part of the ganglion of the same segment. The structure of this epidermal thickening is difficult to elucidate. Two kinds of cell are readily distinguished within it (Text-fig. 19 F, G): (i) A group of eight or nine large cells, with pale nuclei, connected by very fine filaments with the chitinous floor of the depression, within which the base of the seta is lodged (Text-fig. 19 G). It is probable that these cells are the sensory cells of the organ; I cannot, however, recognize any connexions of these cells with the almost imperceptible nerve-fibrils of the sensory nerve. (ii) Numerous small cells, with more deeply staining nuclei, some of which form a peripheral investing layer, while others form a central core of cells. From the latter a

cytoplasmic extension can be followed into the base of the seta. These smaller cells are probably not sense-cells; those which form the central core are evidently trichogen cells.



TEXT-FIG. 19. Structure and development of the trichobothrium.

A. From late embryo. B. From an early pupa, showing initial stage in formation of seta. C. From a later pupa. D. From a very advanced pupa; chitinization has begun, and the expanded base of the seta is forming, the base being occupied by several of the core of trichogen cells. E. From a newly emerged larva; note vestiges of trichogen cells within base of seta. F, G. From an adult animal, the sections being transverse and longitudinal respectively; in G as in E only the base of the seta is indicated.

Lettering. *b.s* base of seta; *c* chitin; *i.c* investing cells; *n* nerve; *s* seta; *s.c* sense-cells; *tr.c* trichogen cells.

The rudiments of the trichobothria first become manifest on the tenth day, as deeply staining aggregations of epidermal cells in the newly forming definitive body-wall. Two pairs only are present, on the fifth and third abdominal segments respectively (figs. 29 A, 31, Pl. 3). In sections they appear as prominent thickenings of the ectoderm that intrude into the underlying yolk (fig. 94 B, Pl. 8; fig. 106, Pl. 9). Almost from the beginning the cells of the thickenings show a tendency to radiate inwards from a point on the surface (fig. 94 B, Pl. 8, left side; Text-fig. 19 A), and this is soon followed by the appearance of a cavity within the thickening. Into the cavity there intrudes a gradually lengthening filament, arising from a few rather more deeply staining cells on

its floor. From the filament will develop the seta; the deeply staining cells are trichogen cells. In this condition the developing trichobothrium is found in late embryos and early pupae (Text-fig. 19 B). In rather older pupae we see a greater development of the central core of trichogen cells, a few of which are themselves intruding into the cavity, but the sense-cells are not yet distinguishable (Text-fig. 19 C). The latter do not, indeed, become apparent till late in the pupal period, by which time the nerve has become recognizable (Text-fig. 19 D). There is now a very marked distinction between the sense-cells and the outer layer of the investing cells, while the central core of trichogen cells is also conspicuous. Of the latter, a few have clumped together to form the expanded base of the seta, on the surface of which chitin has begun to form. The seta itself has grown in length, and has coiled up beneath the pupal cuticle. In newly emerged larvae the vestiges of the cells within the expanded base of the seta are still to be seen as rounded, heavily staining clumps of degenerate chromatin (Text-fig. 20 E), but these soon disappear, leaving only a short stretch of cytoplasm from the core of the trichogen cells to enter the base of the seta.

(iii) *The Pseudoculi.* These large and problematical organs were first described by Lubbock (1868) as eyes. It is now recognized that in their structure they present little in common with visual organs, and they are usually spoken of as pseudoculi. Their general features suggest some sensory organ. Kenyon (1895) was unable to detect any nerve passing to them, but Silvestri (1902) states that they are connected by a nerve with the deutocerebrum. They present little scope for embryological investigation; their structure, however, is well worth examining.

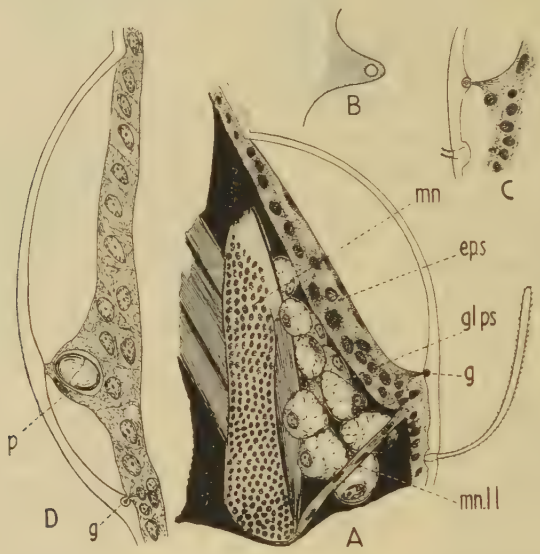
They form a pair of very large, irregularly oval, clear, bulging protrusions of the chitin on the lateral head-wall, perfectly smooth, and free from any hairs or setae (Text-fig. 2 C). Their chitin, which is rather thin, is detached from the epidermis, which does not bulge. Silvestri attributes the glassy appearance of the pseudoculi to air-content; yet in a freshly killed animal, quickly immersed in glycerine, they do not show the familiar total reflexion of an enclosed air-pocket, the space between the epidermis and its bulging chitin being evidently occupied by fluid.

The epithelium of the pseudoculi consists of unusually large epidermal cells; it is mostly one cell in thickness, though in the mid-region they may lie several deep (Text-fig. 20 A). The nerve-fibres from the pseudoculi sweep forward towards the lateral expansion of the deutocerebrum, then curve back and enter the frontal lobe of the protocerebrum (Text-fig. 16 A). Their attachment to the pseudocular epithelium is difficult to observe; they seem to come chiefly from the inner zone of cells at its middle.

When examined in profile, there is seen, intruding into the chitin of the pseudoculus from its hinder margin, a small process of thick chitin from the head-wall (Text-fig. 2 C). Near its tip is a minute spherical globule (Text-fig. 20 B), which proves to be an excavation of the chitin from within. The protoplasm of the adjacent epidermal cells intrudes into the excavation, the

connexion being particularly well seen when the adjacent epidermis shrinks from the chitin (Text-fig. 20 c). This peculiar structure probably serves no other purpose than to prevent detachment of the chitin from its epithelium, along the hinder margin of the pseudoculus.

Of all the known types of sense-organ, the pseudoculus resembles most nearly an organ of hearing. The presence of fluid, rather than air, within it



TEXT-FIG. 20. The pseudoculus and associated structures.

A. Right pseudoculus, viewed from above in optical section; inner end of mandible and pseudocular gland also shown. B. Drawing, under high magnification, of the small globule-bearing process of chitin that intrudes into the hinder margin of the chitin of the pseudoculus. C. Section through same, showing attachment of the epidermis within the globule; the attachment is sufficiently firm to resist the shrinkage which has detached the epidermis from the chitin in the region just behind (below, in drawing) the pseudoculus. D. Section along the left pseudoculus of an undescribed species of *Pauropus*, showing the 'pistil'.

Lettering. *e.ps* epidermis of pseudoculus; *g* globule; *gl.ps* pseudocular gland; *mn* mandible; *mn.l.l* lateral mandibular ligament; *p* 'pistil'.

would probably preclude any effective reception of air-borne sound waves, the 'globulus' of the antenna being probably the organ of hearing in air. But it should be recalled that the Pauropoda live under stones or in decaying wood, in which environment the pseudoculi could probably receive vibrations by direct contact with solid material.

In another (undescribed) larger species of *Pauropus*, sometimes found in small numbers with *P. silvaticus*, the pseudoculus conveys even more vividly the impression of a vibration-receiving organ. Traversing the cavity of the pseudoculus near its middle there is, in this species, a peculiar structure which recalls the 'pistil' described by Silvestri (1920) from *Allopauropus brevisetus*.

It is attached to the inner wall of the pseudocular chitin, and is hollow, its cavity being occupied by a vitreous concretion (Text-fig. 20 D). A firm connexion of this kind might be an effective means of transmitting any motion of the chitin to the underlying epithelium.

The few observations that I have made on the development of the pseudoculi are described in section 10 (v).

(iv) *The Basal Antennal Sense Organs.* Between the bases of the antennae are a pair of very pronounced thickenings of the epidermis, each connected to the deutocerebrum by a thin nerve that lies well above the main antennal nerve (Text-figs. 16, 17). Although located mainly outside the antenna, these epidermal thickenings intrude a little into the basal antennal segment, to the wall of which they are attached (Text-fig. 16 A).

Such an organ might be expected to respond to movement of the antenna as a whole, recalling, in this respect, the peculiar organ of Johnston of the pedicellus of the insect antenna.

They arise as local thickenings of the epidermis in very advanced embryos (fig. 102, Pl. 9). Their nerves probably grow into the brain along the tergal adductor muscle of the antenna, alongside which they lie.

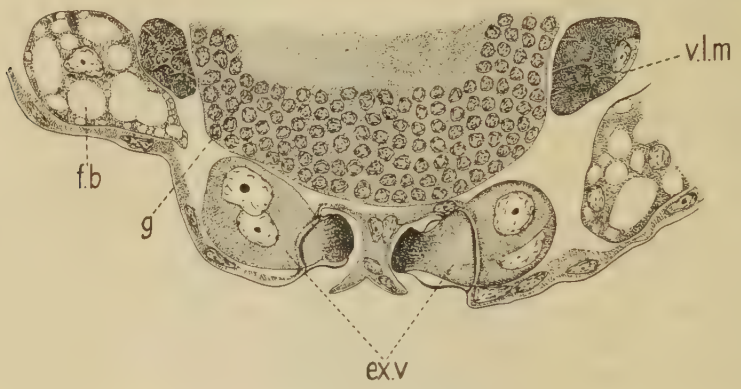
(v) *The Exsertile Vesicles (?) of the Collum Segment.* These peculiar structures are generally regarded, though for no good reason, as the vestigial appendages of the first abdominal segment. Schmidt (1895), who first suggested this, had, however, already recognized their possible affinity with the exsertile vesicles (coxal sacs) of Symphyla.

They are borne on a pair of gentle swellings on the floor of the collum segment, beyond which they protrude towards one another, ending in a slight button-like expansion (Text-figs. 2 B, 24). Each organ appears as a compact bulbous swelling of the epidermis that intrudes well into the body-cavity. It consists of five or six large cells, without obvious cell-boundaries. Their cytoplasm is, for the greater part, granular; but toward the free end it acquires a peculiar hyaline texture, and, unlike the rest of the organ, stains very deeply (Text-fig. 21). The exposed tapering end of the organ is invested in chitin, which becomes thinned out and reduced to a just perceptible sheath on the button-like expansion. The nature of these peculiar structures is not known with certainty. It has been suggested that they may be sense-organs; there is, however, no clearly recognizable nerve connected with them. It seems more probable that they should be compared with the exsertile vesicles of certain other myriapods and primitive insects; but it should be noted that there is no muscle connected with them, though a thin muscle is attached to the chitin immediately to their rear (Text-fig. 17).

They develop from vestiges of the 'ventral organs' of the collum segment, and not out of appendage-Anlagen. In embryos aged about 10 days these 'ventral organs' may be seen in progress of absorption into the collum ganglion, which has itself now separated almost completely from the epidermis (fig. 95, Pl. 8). Sections from more advanced embryos, taken at exactly the same level, show that a small group of cells from each 'ventral organ' has

remained within the sternal epidermis, and from here intrude into the body-cavity (fig. 96, Pl. 8). Out of them the exsertile vesicles will develop. In early pupae the cells and their nuclei are seen to enlarge, and acquire a granular texture of cytoplasm (fig. 97, Pl. 8). Thereafter their lower tapering ends begin to protrude beyond the surrounding epidermis, and form the peculiar button-like flattening above referred to. They now differ from the adult organs only in their smaller size.

The gentle swelling on the floor of the collum segment, on which these organs are borne, may readily be seen in advanced embryos (fig. 30 A, Pl. 3).



Text-fig. 21. Transverse section through floor of collum segment, from a second instar larva, showing structure of 'exsertile vesicle'.

Lettering. *ex.v* exsertile vesicle; *f.b* fat-body; *g* collum ganglion; *v.l.m* ventral longitudinal muscle.

In Symphyla, where there is a succession of well-developed exsertile vesicles along the abdominal segments, their derivation from the 'ventral organs' of the embryo is clear (Tiegs, 1940). The similar origin of the organ of the collum segment of *Pauropus* is additional evidence of affinity with exsertile vesicles, and is conclusive evidence against their supposed homology with appendages, for these arise in a position lateral to the 'ventral organs'.

(vi) *The Coxal Apodemes and the Hypopharyngeal Apophyses.* The coxal apodemes, which are shown in Text-fig. 15, are thin, backwardly directed chitinous ingrowths from the anterior margin of each coxa, and are connected at their hinder ends with intersegmental attachments of the ventral longitudinal muscles. They arise in the advanced embryo as simple epidermal ingrowths from the bases of the coxae, and become chitinized shortly before the larva emerges (fig. 107, Pl. 9).

The structure of the hypopharyngeal hypophyses is described in section 6 (ii) (*b*). They begin to form on about the ninth day, as a pair of ingrowths of epidermal cells, just anterior to the mandibles, and a little median to the pre-mandibular glands. In the absence of intersegmental lines it is impossible to determine accurately their segmental allocation; but their position immediately in front of the mandibles makes it probable that they are actually

of intersegmental origin, having grown in along the intersegmental line between the mandibular and pre-mandibular segments. During the tenth day they grow back into the cavity of the head, where they may be seen in a groove between the pre-mandibular and mandibular ganglia, just median to the ingrowing mandibular apodemes (fig. 115 A, Pl. 9). In sections cut 'horizontally' through the head they are found to have bent round the ganglia on to the (posteriorly directed) superior surface of the latter (fig. 94 D, Pl. 8). In contact with them, at their sides, is the mesoderm of the mandibles.

Before the end of the tenth day the hinder tips of the ganglia have become connected, behind the pre-mandibular ganglia, with the mesoderm cells from the oesophagus; out of these mesoderm cells, which may be seen in fig. 116 B, Pl. 10, will develop the oesophageal dilator muscles. Already at this time the ascending arms of the apophyses have begun to form; the latter may be seen in fig. 116 A, Pl. 10, growing up over the lateral surface of the brain on to the dorsal head-wall, and it will be evident from the section (which immediately precedes fig. 116 B in the series) that the ascending arms develop at only a short distance from the hinder tips of the apophyses.

In the advanced embryo a short string of cells has appeared connecting the mandibular apodemes with the hypopharyngeal apophyses (fig. 117, Pl. 10), but from which of these two structures they have arisen I am unable to state. In them we see the Anlage of the median ligament of the mandible (see section 6 (ii) (*b*)).

In young pupae the apophyses are still two separate cords of cells that bend round the tritocerebrum from their point of origin just in front of the mandibles to their attachment to the oesophageal dilator muscles, and with the ascending arm arising from near their hinder ends (fig. 119, Pl. 10). But as the pupa matures the ganglia recede considerably in the head (cf. Text-figs. 8, 9), enforcing elongation of the apophyses, which now enter the collum segment; and this elongation takes place mainly behind the point of origin of the ascending arms. The oesophageal dilator muscles, hitherto transversely disposed (fig. 117, Pl. 10), are thereby drawn into a position in which they lie more and more parallel with the oesophagus; this is, indeed, already seen in fig. 119, Pl. 10. With the enlargement of the apophyses, their bases approach one another below the oesophagus, where they finally fuse (fig. 120, Pl. 10); in this way the ring round the oesophagus begins to develop.

In these late pupae the apophyses are seen to be continued into two diverging arms of cells that lie in front of the mandibles. From fig. 120, Pl. 10, it is evident that they form part of the wall of the pre-oral cavity, and that they are the suspensorium of the hypopharyngeal apophyses (see section 6 (ii) (*b*)). They should probably be assigned to the pre-mandibular segment.

Shortly before the larva is due to emerge the apophyses develop an inner core of chitin.

(vii) *The 'Dorsal Organ'*. An unpaired 'dorsal organ' is present in the embryo of *Pauropus*, being located in the provisional body-wall mid-dorsally between the anterior and posterior tips of the germ-band.

There is much individual variation in the time of its appearance; the large

cells out of which it will form are sometimes distinguishable as early as the advanced gastrula, while in exceptional cases even late blastoderms are met with in which they are not yet recognizable.

A section through a late gastrula, with the 'dorsal organ' in course of formation, is shown in fig. 46, Pl. 4; a group of five 'yolk-pyramids' (of which two only are present in the section) are here distinguishable by the great size of their nuclei, which, unlike those of the adjacent blastoderm, still lie embedded in the yolk. On the other hand, in the early blastoderm shown in fig. 47, Pl. 4, such cells are not even present. But in advanced blastoderms they may almost always be seen. Below them the intravitelline protoplasmic reticulum is unusually dense, and extends even as far as the central clump of endoderm (figs. 48, 49, Pl. 4; fig. 53, Pl. 5). Throughout the whole course of development of the 'dorsal organ', its cells remain, without cell-walls, in direct continuity with this intravitelline protoplasm.

Probably in consequence of individual differences in the time of first appearance of the 'dorsal organ', sections through the immature organ do not present any uniform picture. Sometimes the large nuclei lie well below the surface of the egg, embedded in yolk (fig. 48, Pl. 4); and here we are probably concerned with an organ which has begun to develop prematurely in the gastrula. In other cases the enlarged cells are a portion of the blastoderm itself, and give no evidence of having arisen within the yolk (fig. 49, Pl. 4). Eventually, however, there is formed, in all cases, a circular disk of enlarged cells which have their long axes towards the middle of the disk (fig. 55, Pl. 5); their cytoplasm is granular and suggests a secretory function. This circular disk of enlarged cells is sometimes, as in the case here illustrated, present as early as the blastoderm stage; but more frequently it does not appear till well after the germ-band has formed.

In this condition the organ does not long remain, for it soon intrudes more deeply into the yolk, while a depression develops at the surface in which a secretion then begins to appear (fig. 56, Pl. 5; Text-fig. 5). This soon increases in quantity, filling the gradually deepening hollow in the middle of the disk, and slowly spreading from there outwards underneath the blastodermic cuticle (fig. 57, Pl. 5).

During the eighth day the organ shows signs of becoming constricted off from the surrounding epidermis (Text-fig. 7; fig. 103, Pl. 9), and eventually loses connexion with the latter, its cells undergoing disruption in the yolk. The secretion is usually visible for a few days longer, spreading for a little distance from the site of the former organ beneath the blastodermic cuticle, to which it probably serves to attach the embryo.

Students of arthropod development have long been familiar with the occurrence of 'dorsal organs' and paired 'dorso-lateral organs' in the embryos of some of the most diverse members of the phylum. There can be little doubt that the organs referred to by this name are not all homologous, and structurally they may differ widely. The Symphyla, Collembola, and *Campodea* are distinguished by the possession of an unusually remarkable organ, for it is the

source from which a system of extra-embryonic filaments radiate, sometimes for long distances, over the surface of the embryo (Tiegs, 1942 *a*, 1942 *b*). The presence of similar organs in the embryo of *Pauropus* would not have caused surprise; as it has turned out, however, the most singular feature of this type of organ—the system of extra-embryonic filaments—is lacking. Neither in Diplopoda nor Chilopoda is a 'dorsal organ' of this type known, and it may, for the present, be regarded as limited to Symphyla and primitive insects.

The 'dorsal organ' of *Pauropus* recalls, in one respect, the paired 'dorso-lateral organs' of certain crustacean embryos. In *Idotea* these organs have a glandular function, their secretion probably serving to attach the embryo to the egg-shell (Nusbaum and Schreiber, 1898). In *Hemimysis* the organ is also secretory (Manton, 1928). In both cases it is purely transitory, and degenerates within the yolk.

15. *The Muscular System*

(*a*) *Musculature of Adult*

Silvestri (1902) has already given a good account of the principal muscles of *Allopauropus brevisetus*. His description, however, omits the muscles of the mouth-appendages, on which information is specially needed. For the purpose of the present work I have made a more complete examination of the adult musculature, which on the whole has confirmed Silvestri's work.

The tergal muscles of the head (Text-fig. 22 B, D) comprise: (i) Two long muscles (*t.ab.a*, *t.ad.a*), which pass forward from the occipital suture, and are attached to the base of the antenna, on which they exert an abductor and an adductor function respectively; (ii) a very large dorsal-longitudinal muscle (*d.l.m.h*), lying lateral to these muscles, originating from the occipital suture, and attached in front to the wall of the head-capsule, lateral to the antenna; this peculiar muscle has, for its probable function, the adjustment of convexity of the chitin of the pseudoculus; (iii) two large muscles originating from the roof of the head-capsule and inserted on to the mandibular apodeme, one (*t.rt.mn*) running forward, and acting probably as a retractor on the mandible, while the hinder and shorter muscle (*t.d.mn*) is probably a depressor; (iv) a long and narrow levator (?) of the maxilla (*t.l.mx*), taking origin from the occipital suture, and inserted on to the stipes of the maxilla; (v) a narrow retractor (?) of the maxilla, arising from the side of the head, and attached to the maxillary apodeme (*t.rt.mx*; Text-fig. 22 A).

Most of the sternal muscles of the head-appendages take origin from the hypopharyngeal apophyses. They comprise (Text-fig. 22 A, C): (i) Two narrow muscles (*s.r.a*) to the basal segment of the antenna, upon which, according to Silvestri, they may exert a rotator effect; (ii) a strongly developed set of muscles to the mandible, most of which must act as adductors (*s.ad.mn*); (iii) a muscle which takes origin from the suspensory plate (Text-fig. 2 F) lies parallel with the mandible and probably acts as protractor (*s.pr.mn*); (iv) a long and narrow muscle (*s.pr.mx*) arising from the apophysis and attached to the hinder end of the maxillary apodeme, and functioning probably as a protractor. Associated

with the maxilla are at least two small muscles: (i) a flexor muscle passing from the stipes to the lacinia (*f.l*); (ii) a very thin sternal depressor (?) arising from the floor of the head (*s.d.mx*; Text-fig. 22 B).

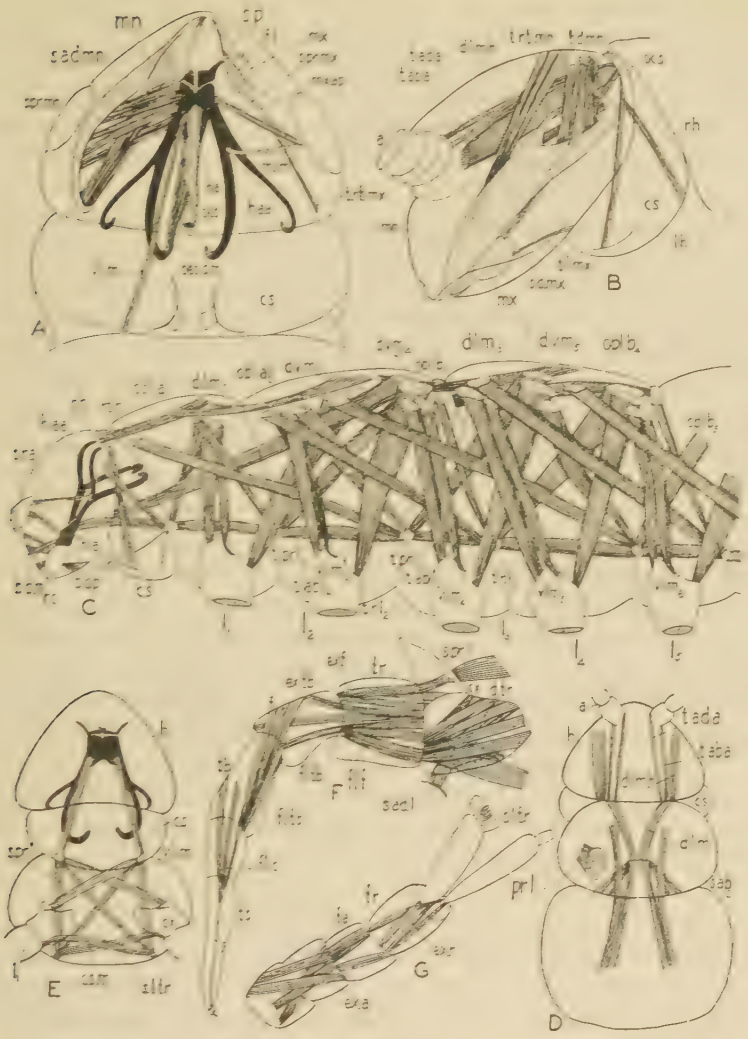
The muscles associated with the fore-gut and pre-oral cavity are (Text-figs. 17, 23 A, C): (i) Two sets (outer and inner) of short buccal dilator muscles (*b.d.m*), the m. elevatores palati of Silvestri, which pass from the roof of the pre-oral cavity to the clypeus; (ii) a pair of muscles that arise from the lateral margins of the intermaxillary plate, and converge on to the floor of the pre-oral cavity, on which they act as depressors (*b.dp*); (iii) a short retractor to the clypeus (*r.c*), arising from the hypopharyngeal apophysis; (iv) a set of very strongly developed oesophageal dilator muscles (*oes.d.m*), which take origin from the hindermost end of the apophysis, and pass forward to become attached to the floor of the oesophagus; the remarkable development of these muscles suggests a strong suctorial function for the oesophagus (see also Text-fig. 16 B).

It should be noted that there are no muscles arising from the dorsal arm of the hypopharyngeal apophysis; this arm is attached by fibrous tissue to the occipital suture, and evidently imparts stability to the apophysis.

The musculature of the antenna (Text-fig. 22 G) comprises a number of short flexor (*f.a*) and extensor (*ex.a*) muscles, passing between the segments, as well as a flexor (*f.r*) and extensor (*ex.r*) muscle for each of the two rami.

The trunk musculature, as might be expected for such active animals, is strongly developed. The following sets of muscles may be identified (Text-fig. 22 C, D, E): (i) the dorsal longitudinal muscle (*d.l.m*), comprising some relatively long muscles that run from the middle of one tergal shield to the middle of the next, as well as some shorter slightly diverging muscles that pass forward or backward from the middle of one tergite to the posterior or anterior margin respectively of the adjacent tergites (see Text-fig. 22 D); (ii) a set of oblique abdominal muscles (*obl.a*) which pass downwards from the anterior margins of the tergal shields, and become attached at the intersegments, one or two segments to the rear; (iii) a set of oblique abdominal muscles (*obl.b*) which pass upwards from their intersegmental attachments, and become connected to the hinder margins of the tergal shields, usually of the succeeding segment (the most anterior of these acts as a retractor of the head—*rt.h*); (iv) simple dorso-ventral muscles (*d.v.m*) that pass down from the tergal shields to become connected at the intersegments, the fourth, sixth, eighth, and tenth segments, which are devoid of tergal shields, utilizing the preceding tergal shields for the dorsal attachment of these muscles; (v) the ventral longitudinal muscles (*v.l.m*), segmentally disposed, and attached at successive intersegments, the most anterior of this set being attached to the hypopharyngeal apophyses; (vi) a set of crossed sternal muscles (*c.s.m*), which pass back from one intersegment to the succeeding intersegment of the other side (Text-fig. 22 E).

Apart from these muscles there are also three large muscles that exert an action on the head (Text-fig. 22 C). These are: (i) a rotator (*r.h*) arising from



TEXT-FIG. 22. Muscular System.

A. Head and collum segment, viewed from below; on the left side has been drawn the mandible, on the right the maxilla only. B. Head and collum segment in lateral view. C. Trunk musculature, lateral view; the numerals attached to individual trunk-muscles are for comparison with Text-fig. 25. D. Tergal muscles, viewed from above. E. Sternal muscles, viewed from below. F. Right leg, in ventral view. G. Antenna. The key to the lettering of the muscles is given in the text.

Additional lettering: *a* antenna; *c.s* collum segment; *cx* coxa; *f* femur; *h* head; *h.a* hypopharyngeal apophysis; *h.a.a* ascending arm of latter; l_1–l_5 first to fifth legs; *m.l.m* median 'ligament' of mandible; *mn* mandible; *mx* maxilla; *o.s* occipital suture; *oes* oesophagus; *s.ab*$_2$ second abdominal segment; *s.p* suspensorial plate; *tb* tibia; *ts* tarsus; *tr* trochanter.

the hinder margin of the collum segment; (ii) a levator (*l.h*) arising from the floor of the collum segment (see also *l.m.h*, Text-fig. 17); (iii) a long and powerful retractor (*rt.h*), arising from the hinder margin of the second tergal scute, and attached to the hinder margin of the head (this muscle is, as Text-fig. 22 C shows, the first member of the second oblique series); (iv) two pairs of thin muscles passing to the floor of the collum segment (shown in Text-fig. 22 C, but not labelled).

The extrinsic musculature of the leg comprises the following muscles (Text-fig. 22 C, E): (i) three tergal muscles for each leg, namely, a large pro-motor (*t.pr.l*), a weaker abductor (*t.ab.l*), and an exceptionally large backwardly directed re-motor (*t.r.l*); (ii) two sternal muscles arising intersegmentally from the opposite side, one muscle being attached to the trochanter of the leg and acting evidently as a levator (*s.l.tr*), while the other muscle is attached to the apodeme of the coxa, and is probably a pro-motor of the leg (*s.pr.l*); (iii) a short adductor (?) from the apodeme to the coxa (*s.ad.l*) (Text-fig. 22 F).

The intrinsic muscles of the leg have already been described by Silvestri (1902) and Ewing (1928). Both these authors have shown that there are no muscles arising within the last three segments of the leg, which, following Ewing, we may therefore regard as tarsus. The muscles of the leg are shown in Text-fig. 22 F. The principal muscles of the coxa comprise a strongly developed system of depressor muscles for the trochanter (*d.tr*); I cannot recognize a levator for the trochanter, other than the large sternal levator which arises from the opposite intersegmental apodeme. The remaining muscles of the leg comprise: (i) a strongly developed system of flexors and extensors for the femur (*fl.f*; *ex.f*); (ii) extensor and flexor muscles for the tibia (*ex.tb*; *fl.tb*); (iii) a large flexor for the tarsus (*fl.ts*), an extensor being apparently absent; (iv) a muscle inserted by a long tendon on to the claw, and acting as a flexor of the claw and of the tarsus (*fl.c*).

(*b*) *Development*

(i) *Muscles of the head.* The tergal muscles of the antenna and the dorso-lateral longitudinal muscle of the head arise from the antennary mesoderm. After breakdown of the antennary somite, the hollow of the antenna becomes filled with an unorganized mass of mesoderm cells which, during the ninth day, spreads as an irregular column of cells from the base of the appendage backward along the dorso-lateral head-wall to the side of the brain (fig. 110, Pl. 9). From this mesoderm a mass of cells separates off below the base of the antenna, and becomes the rudiment of the dorso-lateral longitudinal muscle; other cells spreading back along the roof of the head may be seen, in very advanced embryos, in process of conversion into the tergal muscles of the antenna. In newly formed pupae muscle fibres are already distinguishable.

The sternal muscles of the antenna and of the mouth-appendages develop in intimate association with the hypopharyngeal apophyses. The latter, as already described in section 14 (vi), begin to develop during the ninth day as a pair of ectodermal ingrowths, immediately in front of the anterior margin of

the mandibles. They grow backwards into the cavity of the head, where, during the tenth day, they may be seen lying to the side of the mandibular ganglion. Their relation to the latter, and to the mesoderm of the antennary and gnathal segments, will be understood by reference to figs. 94 D and 115 A, B, Pl. 9, fig. 115 being drawn from a 10-day embryo, and representing a frontal section through the head, while fig. 94 D is a 'horizontal' section through a similar embryo, just above the bases of the mandibles. It will be seen that each apophysis lies just median to the main mass of the mandibular mesoderm, which is being drawn into the cavity of the head with the ingrowing mandibular apodeme. Mesoderm cells from the hinder margin of the base of the antenna lie in close proximity, a little dorso-laterally to the mandibular apodeme, while from below mesoderm from the maxillary segment is growing upward. The relation of the latter to the apophysis is not clear from the section shown in fig. 115 A, Pl. 9, but if we examine the section immediately to the rear (fig. 115 B), it is at once evident that maxillary mesoderm is being drawn in with the ingrowing maxillary apodeme, just behind the mandibular apodeme, into the direction of the apophysis.

The apophysis is thus in close proximity to the mesoderm of all three head-appendages. In sections through later embryos we find that a direct connexion between this mesoderm and the apophysis has been established (fig. 112, Pl. 9). Out of this will form the sternal musculature of the appendages, their differentiation into muscle-fibres taking place in the advanced embryo and pupa. The development of the sternal muscles of the antennae is necessarily attended by a considerable change in their direction, as these appendages gradually move on to the anterior surface of the head.

The tergal muscles of the mandibles arise from cells that pass from the hinder tips of the mandibular apodemes on to the head-wall, i.e. purely out of mandibular mesoderm. I have not been able to observe the origin of the tergal muscles of the maxilla. (It must be evident, from the manner of their formation, that the attachment of the tergal mandibular and antennary muscles on the wall of the head cannot provide a guide to defining their segments in the definitive head-capsule.)

The development of the large oesophageal dilator muscles has already been described in section 14 (vi).

(ii) *Muscles of the abdomen.* Except for the system of dorsal longitudinal muscles, the abdominal musculature takes its origin wholly from cells that are released by disruption of the abdominal somites.

The Anlage of the dorsal longitudinal musculature is distinguishable very early, for it never forms part of the mesodermal somites. It has already been referred to in section 7. In 7-day embryos, after the somites have begun to form, a pair of irregular bands of mesodermal cells may be seen along the lateral walls of the embryo, dorso-lateral in position to the row of somites (fig. 64, Pl. 5). As far as I have been able to observe, all the segments from the maxillary to the fifth abdominal contribute to their development. In sections cut 'horizontally' along the embryo, the cells in these bands appear irregularly

clumped, but they do not show any obvious sign of segmentation, such as the somites do (fig. 72, Pl. 6). By the ninth day these cells have usually aggregated into four distinct masses (fig. 73, Pl. 6), though sometimes even in older embryos an irregular alignment of cells still persists (fig. 94B, Pl. 8). The most anterior and least well-defined of these masses lies a little behind the antennary somite, and the hindermost in the fifth abdominal segment. They are evidently the Anlagen of the four dorsal longitudinal muscles of the larva. This clumping of the dorsal longitudinal mesoderm into four masses of cells may not be looked upon as an expression of primary segmentation, equivalent to that of the somites, for all the dorsal longitudinal muscles are not strictly segmental in location (cf. Text-fig. 22 C). In later embryos the muscle-Anlage moves into a still more dorsal position (figs. 106, 107, 109, Pl. 9), and there undergo differentiation into muscle tissue.

The disruption of the abdominal somites during the ninth day is attended by a re-grouping of cells, in which the Anlagen of certain of the abdominal muscles become, for the first time, apparent. Of these the most conspicuous are the ventral longitudinal muscles. They soon become recognizable in each segment as a pair of narrow bands of cells, lying alongside the nerve-cord (fig. 78, Pl. 7). They are present in all the abdominal segments except the anal. In the adult animal the most anterior of these muscles is attached to the hypopharyngeal apophysis (Text-fig. 22 C), and it would therefore seem that the gnathal segments of the head contribute some myoblasts to the ventral longitudinal musculature. In sections through later embryos these muscle-Anlagen are exceedingly prominent to the side of the nerve-cord, where they are now undergoing conversion into muscle tissue (figs. 106, 107, Pl. 9). They have by this time acquired intersegmental attachments. In pupae they are seen in a more ventral position relative to the nerve-cord (fig. 108, Pl. 9; also fig. 109, Pl. 9, from a larva).

Spreading down from the base of each appendage, under the ventral longitudinal muscle, is another group of mesoderm cells, out of which the sternal musculature will develop (fig. 78, Pl. 7). In the advanced embryo these cells grow under the nerve-ganglia, after these have separated from the ventral epidermis; their conversion into muscle-fibres begins in the late embryo, even before the pupa forms.

The various dorso-ventral and oblique muscles of the abdomen all arise from cells which spread up from the bases of the legs over the lateral epidermis. They may be seen in fig. 106, Pl. 9. Their conversion into muscle-fibres takes place in the advanced embryo (fig. 107, Pl. 9).

The musculature of the legs develops out of the clumps of mesoderm that occupy the hollows of the limb-buds, becoming drawn out with the latter when, in the late embryo, these elongate to form the legs (fig. 106, Pl. 9).

DESCRIPTION OF PLATES

Plate 1

Fig. 1. Fragment of adult ovary, cut in 'horizontal' section. The germarium lies between the two rows of oocytes that are enlarging along the sides of the ovary. Three of its cells (indicated by *x*) are in prophase of meiosis; one (*y*) is in mitosis; others (*z*) are in resting phase. But within the germarium are also young oocytes, in an initial phase of enlargement, and with nuclei in the germinal vesicle condition. ×770.

Fig. 2. Fragment of a sagittal section along ovary, in which some of the oocytes have begun to accumulate yolk. There are also three enlarging oocytes still without yolk, and in one of these a 'yolk-nucleus' is seen. ×390.

Fig. 3. Polar view of the chromosomes, from an ovarian egg shortly before laying; first meiotic metaphase. The full set of 13 bivalent chromosomes ('tetrads') is shown. ×1,600.

Fig. 4. Similar stage, showing spindle fibres. Only 6 of the 13 bivalents are present in the section. ×1,600.

Fig. 5. From an almost newly laid egg. The chromosomes and their enveloping cytoplasm have moved to the periphery. Nine of the 13 bivalents, still showing 'tetrad' formation, and still located in a single plane, are present in the section. ×1,600.

Fig. 6. Anaphase of first meiotic division. ×1,600.

Fig. 7. The same; polar view. ×1,600.

Fig. 8. Fragment of a tangential section at the surface of the egg, showing first and second polar bodies; from an egg in which fusion of male and female pro-nuclei has already taken place. ×1,600.

Fig. 9. Portion of a section through an egg, showing male and female pro-nuclei just prior to fusion. ×950.

Fig. 10. Section through egg after fusion of pro-nuclei. A true resting nucleus has formed. ×710.

Fig. 11. Egg with 2 blastomeres. ×480.

Fig. 12. Egg with 4 blastomeres. In this egg the cleavage grooves between the blastomeres are unusually deep. ×480.

Fig. 13. Egg with 6 blastomeres. ×480.

Fig. 14. Egg with 8 blastomeres. ×480.

Fig. 15. Egg with 16 nuclei, but in which only 11 blastomeres have so far become demarcated. ×480.

Fig. 16. Egg with 24 blastomeres (early blastula stage). ×480.

Fig. 17. Egg with about 40–5 cells (late blastula). ×590.

Fig. 18. Egg with about 80 blastomeres (early gastrula). ×590.

Fig. 19. Egg with about 200 cells (late gastrula). ×700.

Plate 2

All drawings in this plate are from stained whole embryos.

Fig. 20. Mature blastoderm. ×380.

Fig. 21. Early phase in the differentiation of the germ-band out of the blastoderm; stage with ventral aggregation of nuclei and cytoplasm. ×380.

Fig. 22. Early germ-band; lateral view. ×380.

Fig. 23. Oblique view of an embryo rather more advanced than that shown in the previous figure. The head-lobes have begun to form, and the stomodaeal opening has appeared. ×380.

Fig. 24 A, B. Lateral (left) view and anterior view respectively of a 6-day embryo. The antennae are developing; the stomodaeum is now conspicuous. ×380.

Fig. 25 A, B. Similar views of a 7-day embryo. The rudiments of another pair of appendages (mandibles) have formed. On the head-lobes the invaginated posterior lobes of the protocerebral ganglia have appeared. ×380.

Fig. 26 A, B. Lateral (right) and anterior views of a more advanced 7-day embryo, in which the appendage rudiments as far back as the first legs have appeared. ×380.

Fig. 27 A, B. Similar views of an embryo aged about 8 days, showing two well-developed leg-rudiments, and the third in course of formation. The embryo shows the initial stage in development of the pre-oral cavity. ×380.

Plate 3

Fig. 28 A, B. Lateral (left) and anterior views, respectively, of a 9-day embryo. × about 420.

Fig. 29 A, B. Lateral (right) and antero-ventral views respectively of an embryo aged fully 10 days. Fig. A × 430; Fig. B × 380.

Fig. 30 A, B. A. Antero-ventral view of an embryo, shortly before hatching.

B. View into the pre-oral cavity of the same embryo, to show the inturned mandibular sternite; the optical section through the clypeus is represented as a cut edge by lines.

A and B × 380.

Fig. 31. Postero-dorsal view of the hinder end of an advanced embryo, at about the stage shown in Fig. 29. In this embryo the outlines of the anal segment have become defined. × 380.

Fig. 32 A, B, C. A. Early pupa (pupal sheath removed). × 370.

B. Right antenna of same, dorsal view. × 800.

C. Second leg. × 610.

Fig. 33 A, B. Advanced pupa. In A is shown the anterior end, seen from the right side, × 600. In B is shown the right antenna in ventral view. × 800.

Fig. 34. Hinder end of a newly emerged first instar larva; lateral view. This drawing, and Figs. 35, 36, 37, are from stained larvae. The investing chitin is shown in outline only. Of the setae, only the large setae of the fifth segment (second trichobothria) are indicated. × 470.

Fig. 35. Hinder end of a more advanced first instar larva, in which the fourth and fifth legs are visible beneath the chitin. The new (seventh) segment is in process of forming; note the Anlage of its sensory seta (trichobothrium). × 500.

Fig. 36. Hinder end of a larva of about similar age, seen from above. × 470.

Fig. 37. Hinder end of an advanced first instar larva shortly before the moult. The fourth and fifth legs are now fully developed; in the new (seventh) segment the new (third) trichobothrium has grown out beneath the chitin, but is not yet fully chitinized. The chitin of the second trichobothrium is being discarded, and a new second trichobothrium is replacing it. × 470.

Plate 4

Fig. 38. Section through an egg in which the first two cleavage-nuclei have appeared, but in which the partition separating the blastomeres has not yet formed. Note the spindle-fibres between the daughter-nuclei.

Fig. 39. 'Horizontal' section through the 4-cell egg shown in Fig. 12.

Fig. 40. Section through 8-cell egg shown in Fig. 14. Note degenerating polar bodies.

Fig. 41. Section through an egg containing 11 nuclei, of which 7 are in mitosis. Only 4 nuclei are present in this section. Note degenerating polar body within one of the blastomeres.

Fig. 42. Section through 24-cell egg shown in Fig. 16.

Fig. 43. Section through the egg shown in Fig. 17, with 40–5 cells (late blastula stage).

Fig. 44. Section through developing gastrula; about 75-cell stage.

Fig. 45. Section through young gastrula; about 80-cell stage (cf. Fig. 18).

Fig. 46. Section through an advanced gastrula at about the stage of development shown in Fig. 19. This gastrula shows precocious 'dorsal organ' formation.

Fig. 47. Longitudinal section of a young blastoderm.

Figs. 48, 49. Sagittal sections of two mature blastoderms, showing different conditions of development of the 'dorsal organ'. The blastoderms also show the initial stages of migration of yolk-cells into the yolk.

Plate 5

Fig. 50. Small fragment of a section grazing the surface of a mature gastrula. The drawing represents a transverse section through the outer wall of a single 'yolk-pyramid'. × 800.

Fig. 51. Similar section, from a mature blastoderm. × 800.

Fig. 52. Section through the egg shown in Fig. 21. The section is taken 'horizontally' through the region of thickening, a little below the equator of the egg. Separation of the mesodermal cells from the anterior (to right) and posterior thickened walls is in progress; the intervening thick epithelium at the sides of the egg does not contribute to the formation of mesoderm. Although endoderm is present in the middle of the egg, neither of its two nuclei lies within the section. × 550.

Fig. 53. Sagittal section of an egg at about the stage shown in Fig. 21. Mesoderm formation is here more advanced than in the egg shown in Fig. 52, for the mesoderm already constitutes a fairly well-defined layer internal to the ectoderm in the lower half of the egg. × 550.

Fig. 54. Sagittal section through an egg with newly formed germ-band (cf. Fig. 22); anterior end to right. The mesoderm is now distinct. The stomodaeum has begun to form. Note the peculiar cytoplasmic inclusions in endoderm. × 550.

Fig. 55. Section through a developing 'dorsal organ' from a late blastoderm. × 800.

Fig. 56. Portion of a transverse section through a 6-day embryo, showing 'dorsal organ'. A depression is forming in its middle, and secretion has begun. × 800.

Fig. 57. Similar section. The depression in the organ is now filled with secretion. × 800.

Fig. 58 A, B. Two sections of a 6-day embryo, from a 'horizontally' cut series. Section B passes through the equator of the egg, section A nearer the upper pole. In A the section passes (below) through the head-lobe, in which the protocerebral ganglionic thickenings are developing; and (above) through the developing proctodaeum. In B it passes (below) a little behind the stomodaeum and (above) through the region of the third abdominal segment. × 800.

Fig. 59. Portion of a section from a 'horizontally' cut series, from a rather later embryo than the foregoing. The section passes through the stomodaeum, and includes the antennary somite, which now lies in line with the stomodaeum. × 800.

Fig. 60. Part of a section through the head of an 8-day embryo at level of antenna (cf. Fig. 67 C, D). The antennary 'ventral organ' has begun to form. In the antennary somite a coelomic cavity is now present. × 800.

Fig. 61. Part of a transverse section through a 7-day embryo, a little behind the stomodaeum, to show the pre-mandibular mesoderm. × 800.

Fig. 62. Fragment of a section from an early 8-day embryo, showing pre-mandibular somite beginning to elongate; the large mass of cells to the right is the inferior wall of the pre-oral cavity. × 800.

Fig. 63. Transverse section through maxillary segment of a 7-day embryo, showing initial stage in formation of the somites. Between the developing somites the 'median mesoderm' is visible. × 800.

Fig. 64. Transverse section of right half of a late 7-day embryo, taken through the maxillary segment. Note, in comparison with Fig. 63, that the maxillary somite has taken form, and is now widely separated from the 'median mesoderm'. Note also the Anlage of the dorsal longitudinal muscle. In the ectoderm we see the first indication of ganglion formation. × 800.

Fig. 65. Transverse section through the collum segment of a 7-day embryo, showing initial stage in formation of somites. Between the two developing somites is a little 'median mesoderm'. Drawn from the same embryo as Fig. 63. × 800.

Fig. 66. Similar section from an 8-day embryo, showing fully formed somite of the collum segment. Note the Anlage of the dorsal longitudinal muscle, quite distinct from the somite. Associated with the collum ganglion is a 'ventral organ'. × 800.

Plate 6

Fig. 67 A–F. Drawings of six successive sections cut 'horizontally' through an 8-day embryo, to show the somites and developing ganglia of the head. The pre-oral cavity is developing, and it is not possible to delimit it sharply from the stomodaeal ingrowth. In Fig. B the complete section has been drawn, and shows (above) the abdomen transected through its fourth segment; the other five sections show only the head of the embryo. × 870.

In Fig. A the section passes just in front of the stomodaeum and developing pre-oral cavity, and shows the pre-antennary somites which have arisen from the pre-oral mesoderm; to the sides are the pre-antennary ganglia, and to the sides of these the lateral and frontal lobes of the protocerebrum, in which 'ventral organ' cell-disposition is apparent (a fragment of the posterior lobe of the protocerebrum also intrudes into the section).

In B the section grazes the hinder wall of the pre-antennary somites and the anterior wall of the stomodaeum; note the antennary ganglion lying immediately behind the pre-antennary ganglion of the previous section; note also the antenna, and the intrusion of part of its somite into the section.

In C more of the stomodaeum and developing pre-oral cavity appears, and to its right a fragment of the pre-mandibular somite intrudes into the section; the antennary ganglia are present, the left showing 'ventral organ' cell disposition; more of the antennary somite is present.

In D the section passes along the whole length of the stomodaeum and pre-oral cavity, the stomodaeum having extended to the central clump of endoderm; to the side of the pre-oral cavity are the elongating pre-mandibular somites, and, lateral to these, we now see the main bulk of the antennary somite.

In E the elongating pre-mandibular somites are seen, bending round behind the pre-oral cavity; on both sides the mandibular somites have begun to intrude into the section, and on the right side is seen also the tip of the elongating maxillary somite.

In F the main mass of each mandibular somite is now present in the section, and part also of the maxillary somite, which is in process of elongating into the maxillary gland. The hindermost tip of the pre-mandibular somite intrudes into the section. The large mass of developing ganglion with its 'ventral organ' is the pre-mandibular ganglion. The small clump of ganglion tissue above it is probably mandibular ganglion, intruding from behind into the section (for orientation of the section see Fig. 80).

Fig. 68. Section through the head of an embryo aged about 8 days, taken just in front of the stomodaeum (from a 'horizontally' cut series). The pre-oral mesoderm forms a thick clump of cells, but has not yet developed into pre-antennary somites. To the sides are the strong ectodermal thickenings, from which the pre-antennary ganglia and the lateral and frontal lobes of the protocerebrum will arise, but no 'ventral organ' cell disposition has yet appeared. The drawing should be compared with that shown in Fig. 67 A, which represents a comparable section from a rather more advanced embryo. ×870.

Fig. 69. Fragment of a transverse section through the head of a late 8-day embryo, to show beginning of formation of the left mandibular apodeme, and initial phase in transformation of the mandibular somite. To the right a fragment of the developing pre-mandibular gland intrudes into the section, while to the left is seen a part of the maxillary gland. ×870.

Fig. 70. Similar section to the foregoing, taken from a more advanced embryo, showing right mandible and its developing apodeme; the somite has now disrupted into a clump of myoblasts, from which the mandibular musculature will form. In this section the mandibular ganglion is present; note the remains of its 'ventral organ' forming the floor of the pre-oral cavity. ×870.

Fig. 71. Similar section to that shown in Fig. 62, but from a later embryo in which the pre-mandibular somite is in process of conversion into the pre-mandibular gland. The thickened ectoderm to the left is the mandibular ganglion with 'ventral organ'. ×870.

Fig. 72. Portion of a section cut 'horizontally' through the right half of a late 7-day embryo, showing the Anlage of the dorsal longitudinal muscle. At the upper and lower ends of the section a portion of the fourth abdominal and antennary somites respectively may be seen. ×870.

Fig. 73. Similar section from left half of a 9-day embryo. The Anlagen of the four dorsal longitudinal muscles are now seen. ×870.

Plate 7

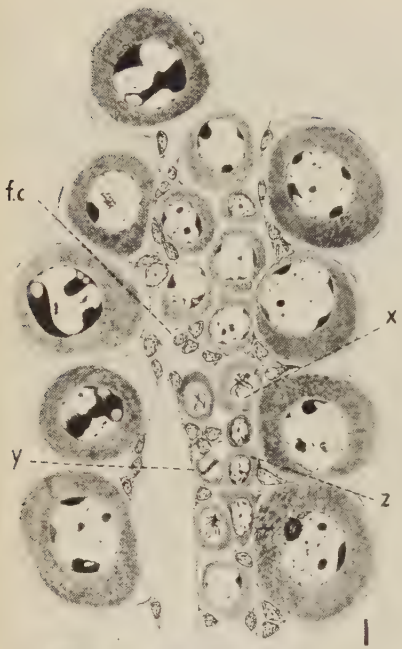
Fig. 74. Transverse section through second abdominal segment of a 7-day embryo, to show initial stage in formation of somites. The section is from the same embryo as are Figs. 63 and 65. ×950.

Fig. 75. Similar section, from a rather older embryo, showing later stage in the formation of the somite. ×950.

Fig. 76. Section through fourth abdominal segment of an embryo a little more advanced than that from which a corresponding section in Fig. 67 B has been drawn. The ganglion-rudiment has enlarged and 'ventral organ' formation has begun. The somites occupy the cavities of the limb bases. In one somite a coelomic cavity is present; the other does not even display a visceral wall. ×950.

Fig. 77. Portion of a longitudinal section along a 9-day embryo, showing beginning of transformation of the somites of the second and third abdominal segments. The somites, still showing a trace of coelomic cavity, are enlarging at their lower ends, and extending into the appendages, of which the bases are present in the section. ×950.

Fig. 78. Section through second abdominal segment of a 9-day embryo. The legs are not present in the section. The somites have disrupted each into a large clump of myoblasts, within which the future ventral longitudinal muscle can already be distinguished. The ganglion is now much enlarged, and four of the ganglion-cells are in mitosis. The 'ventral organs' are at



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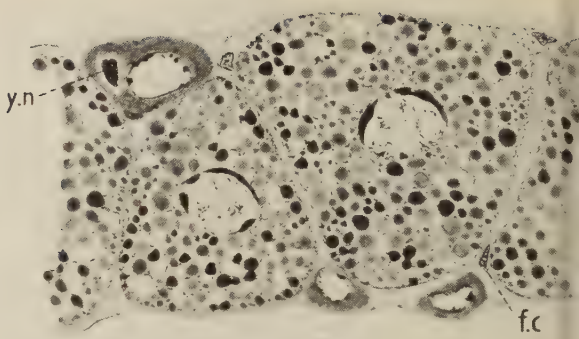

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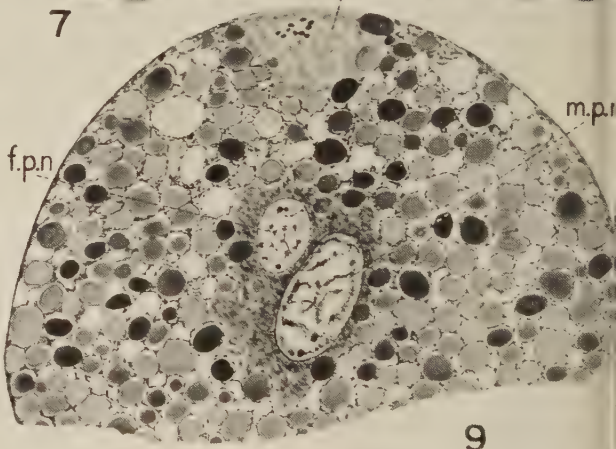

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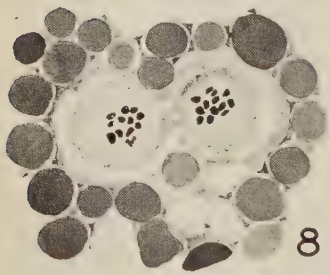

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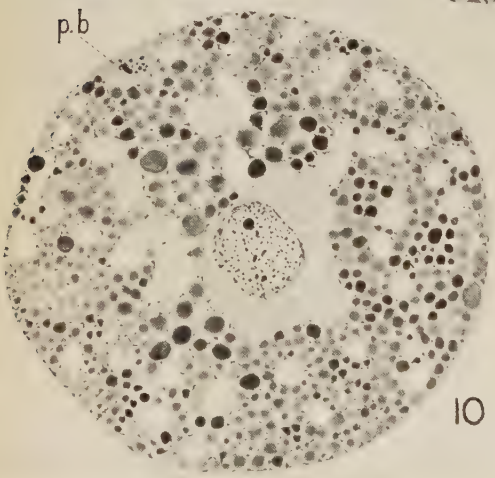

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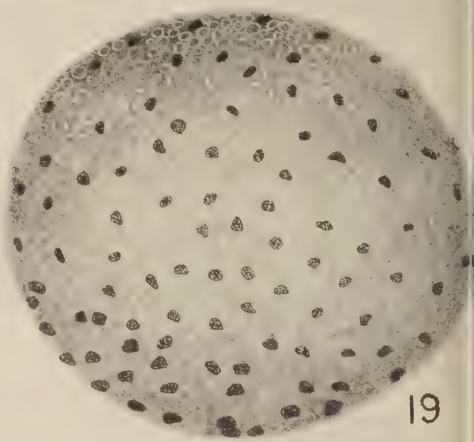

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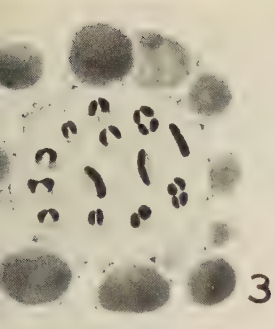
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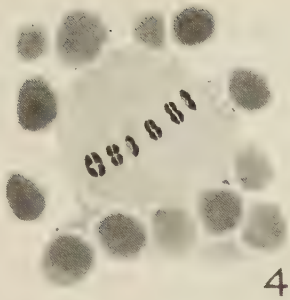
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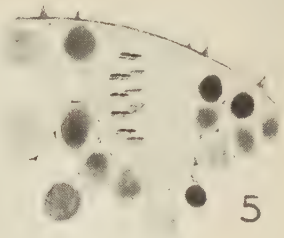
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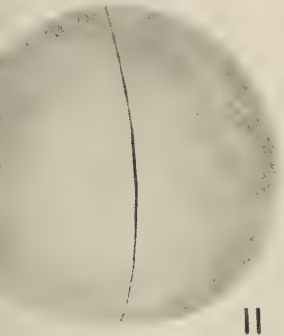
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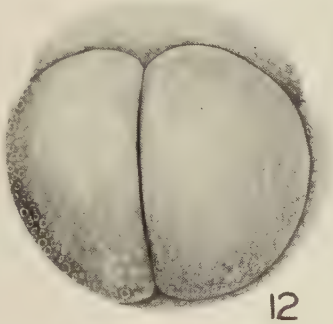
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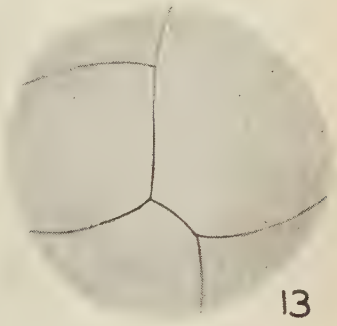
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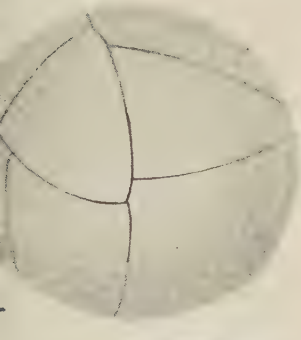

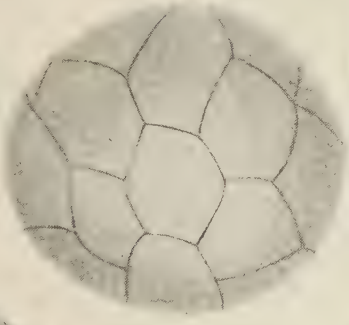
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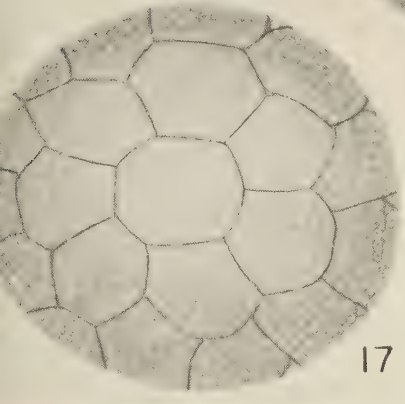
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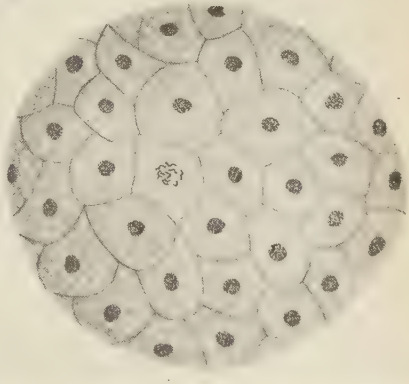
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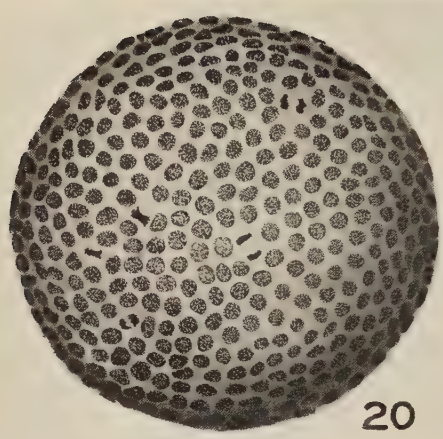

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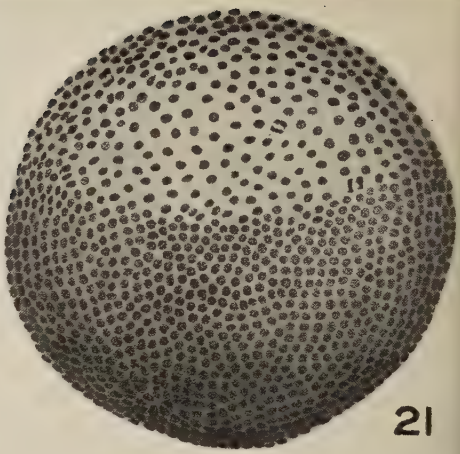

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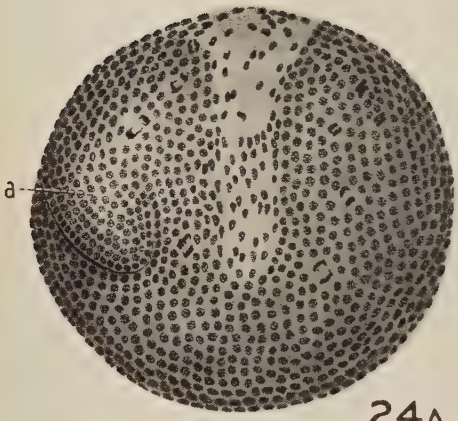

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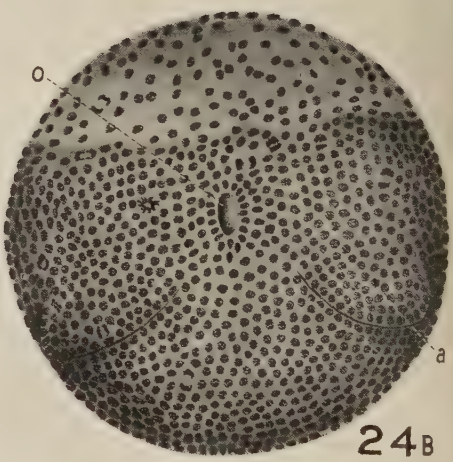

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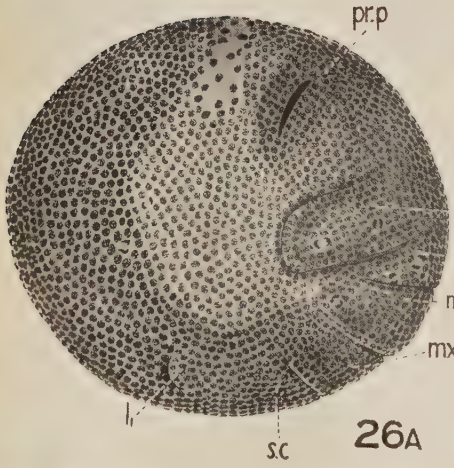

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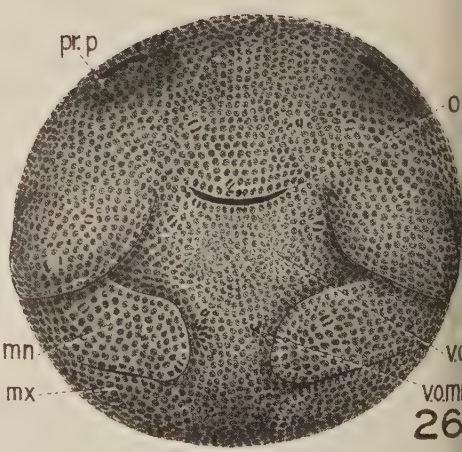

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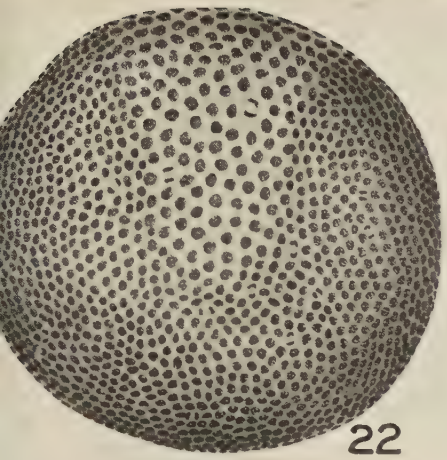

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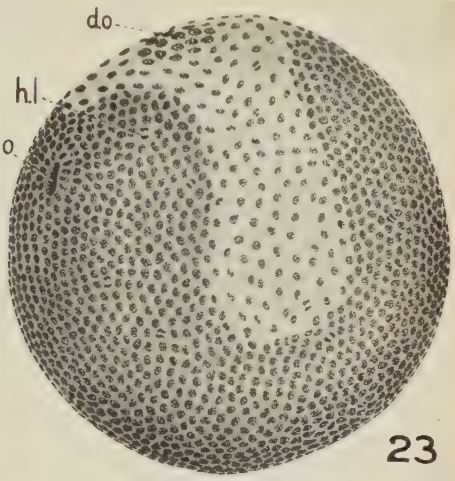

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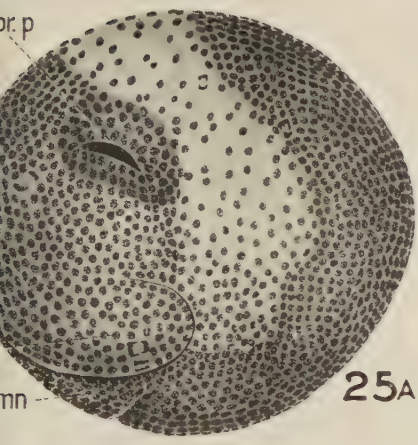

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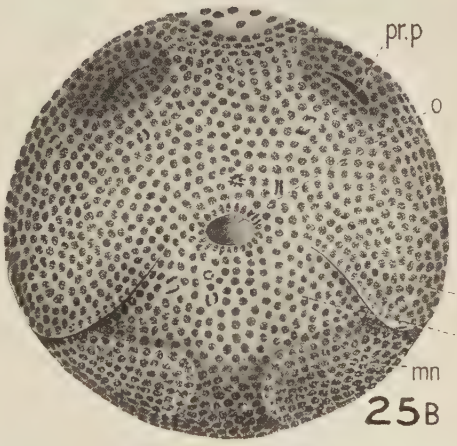

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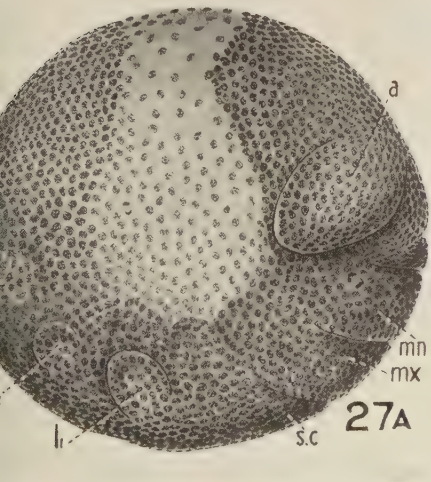

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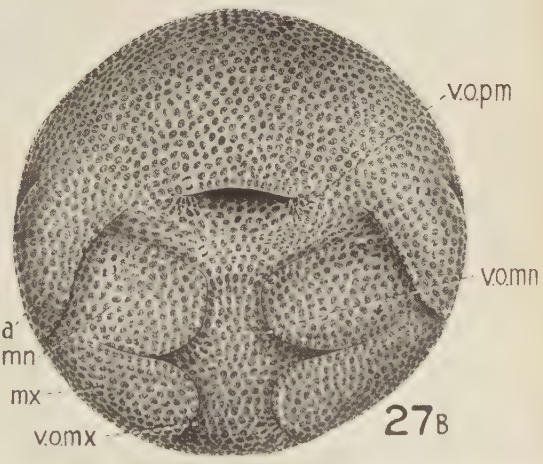

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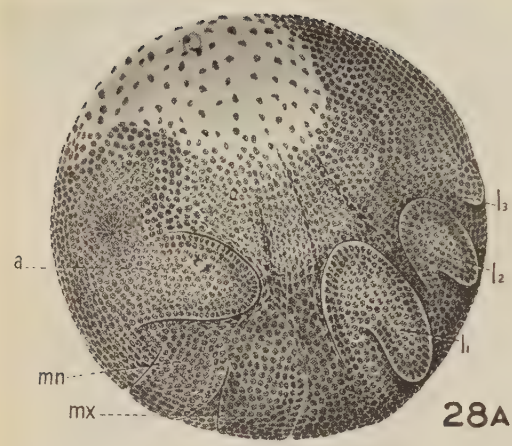

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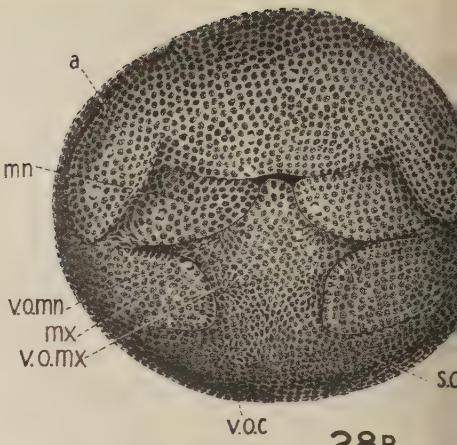

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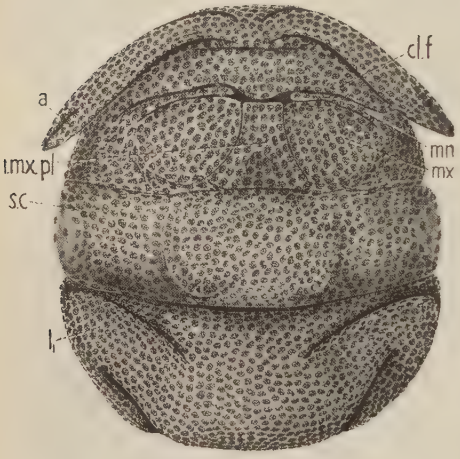

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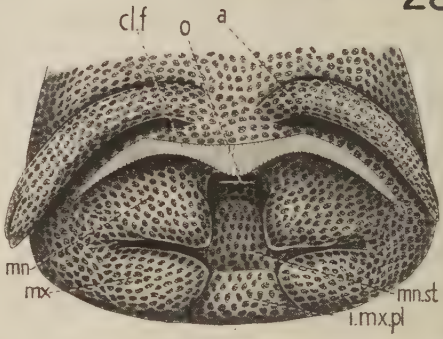

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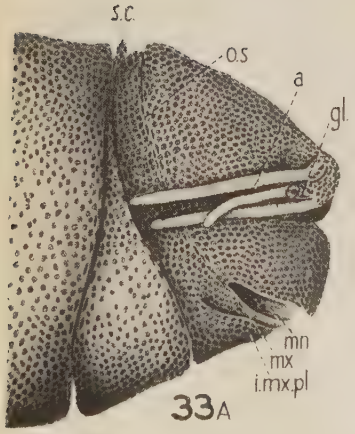

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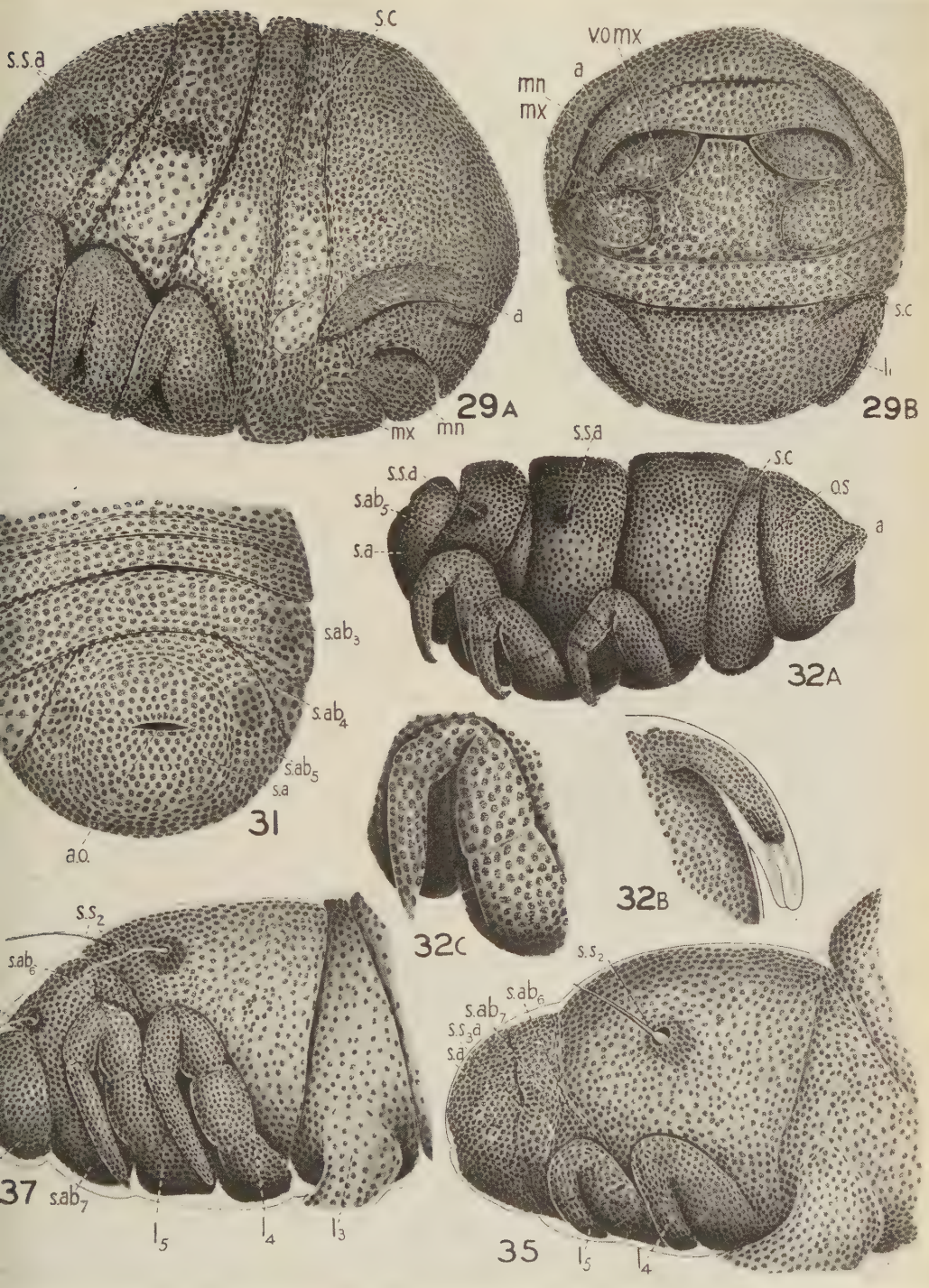
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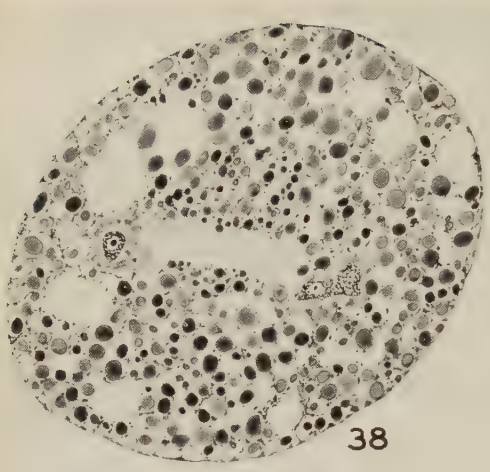

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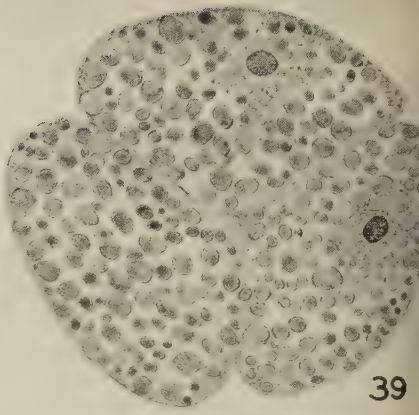

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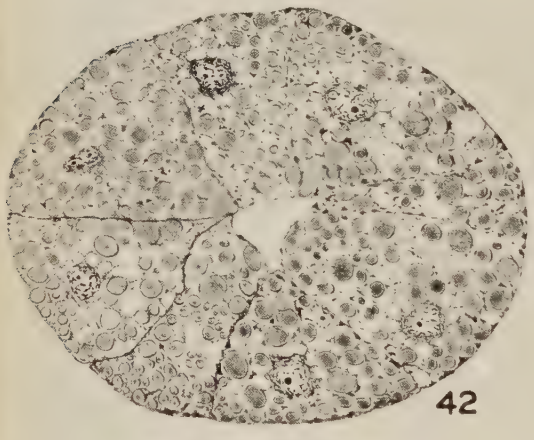

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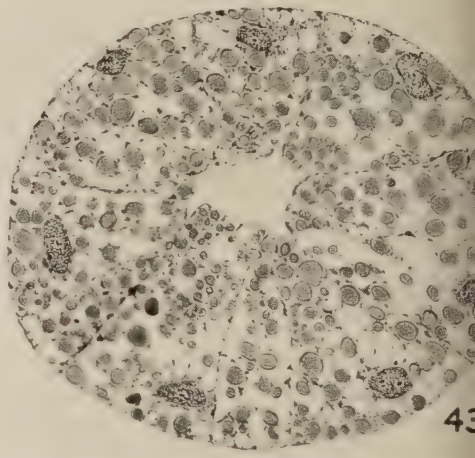

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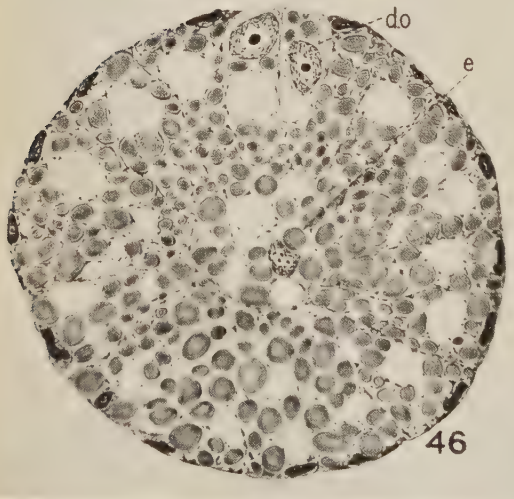

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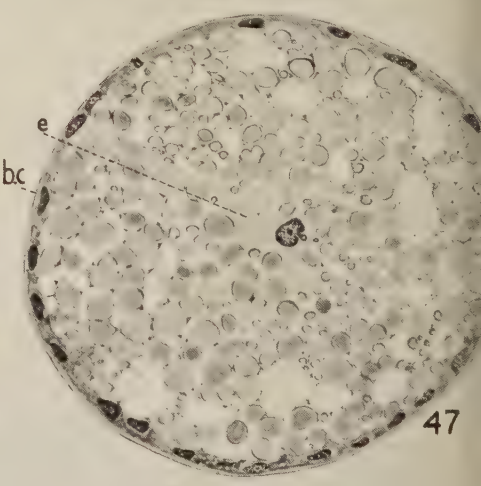

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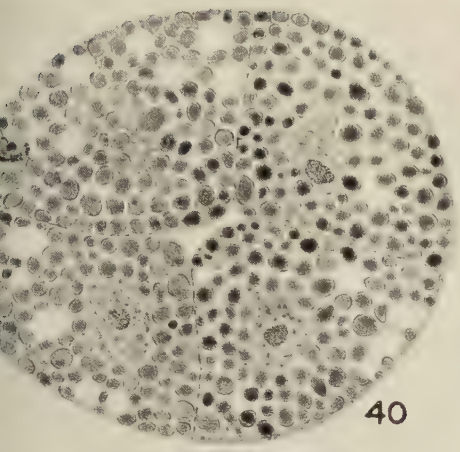
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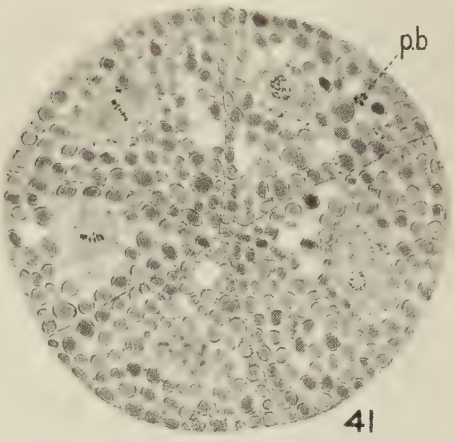
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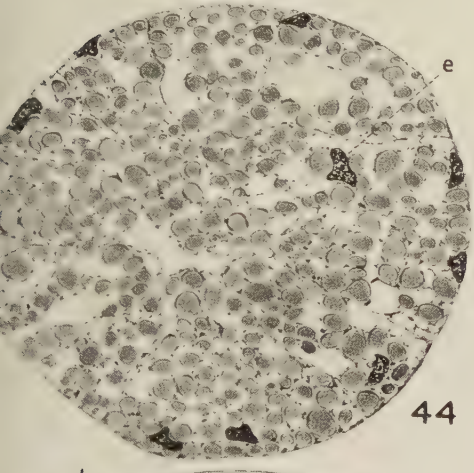
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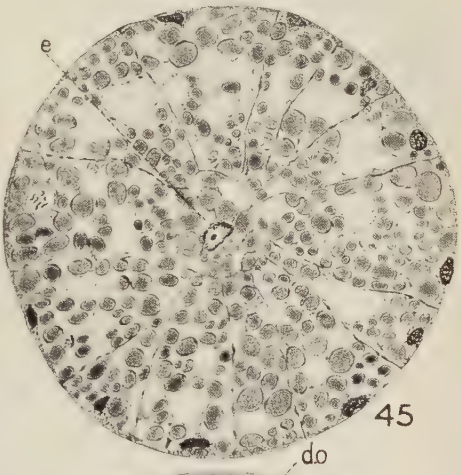
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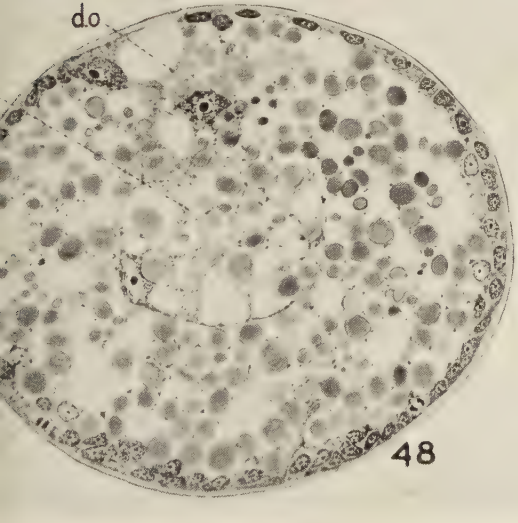
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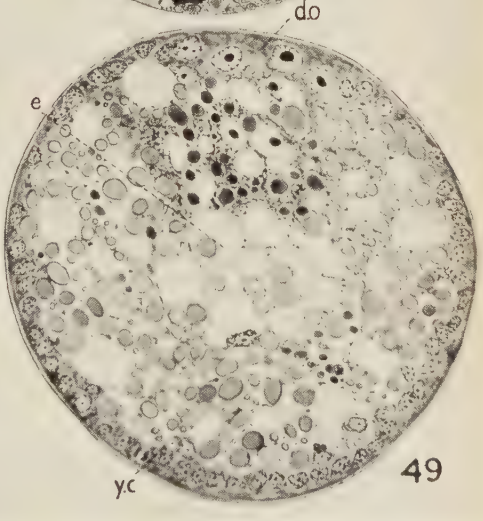
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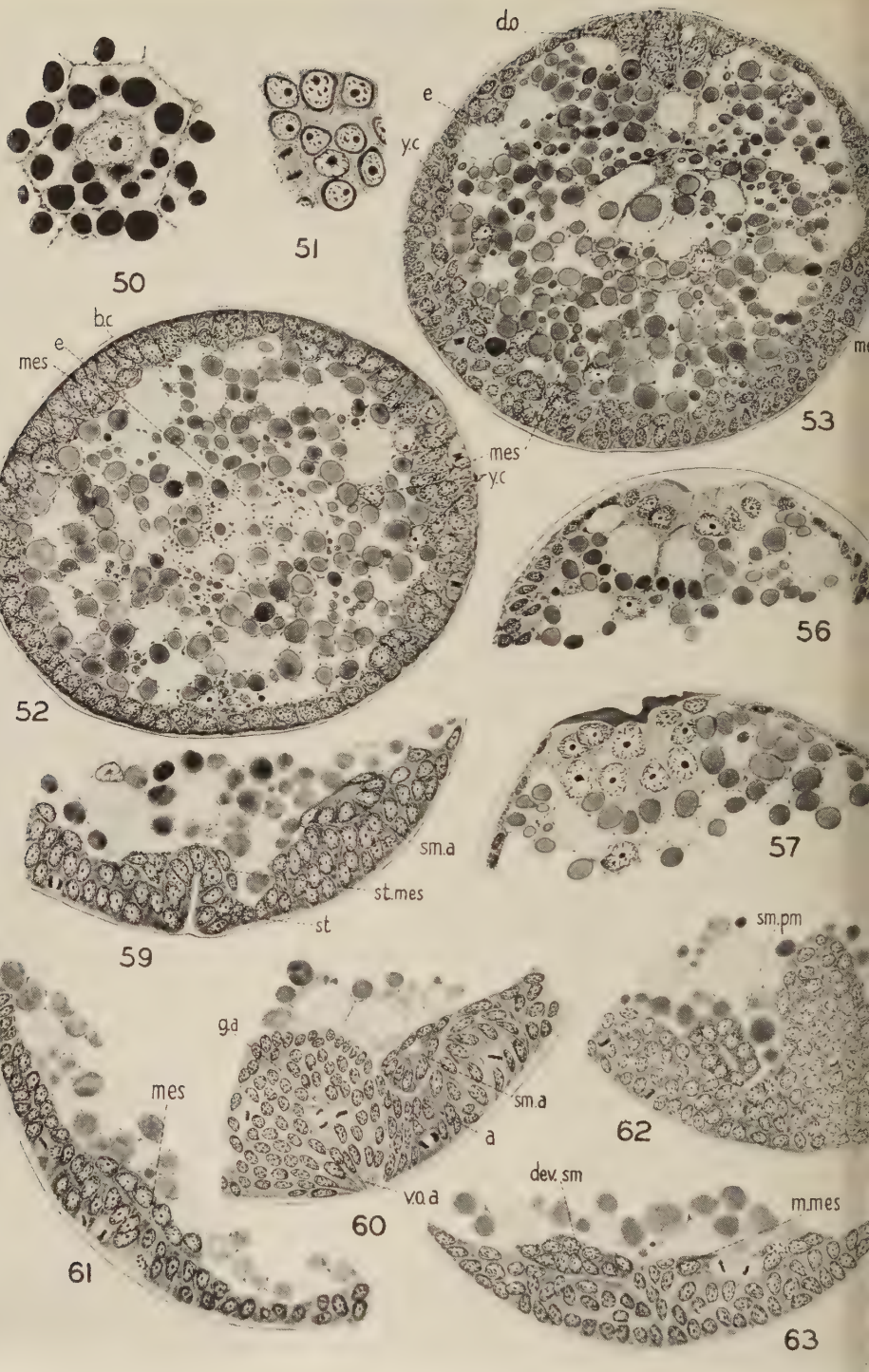
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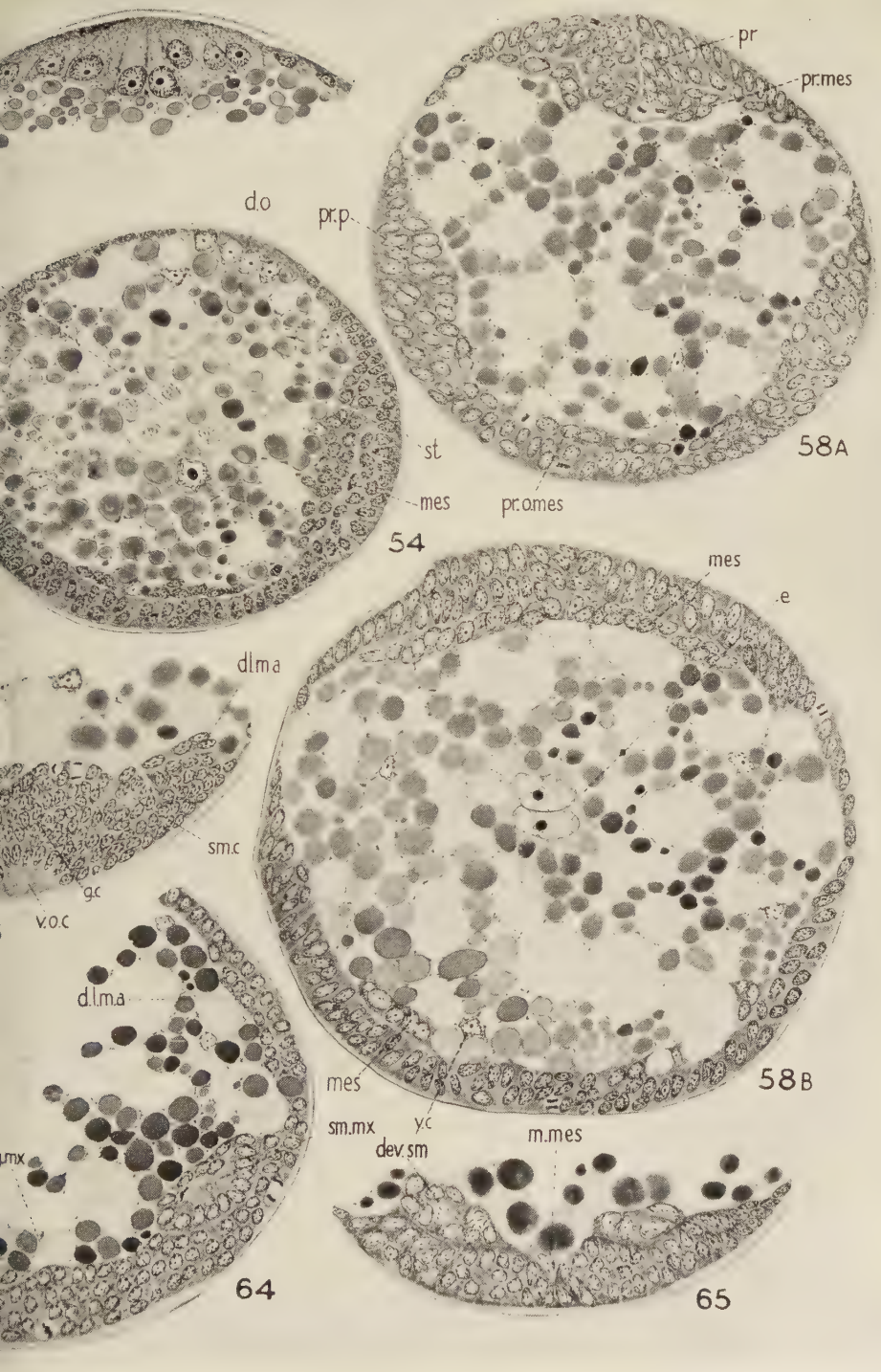
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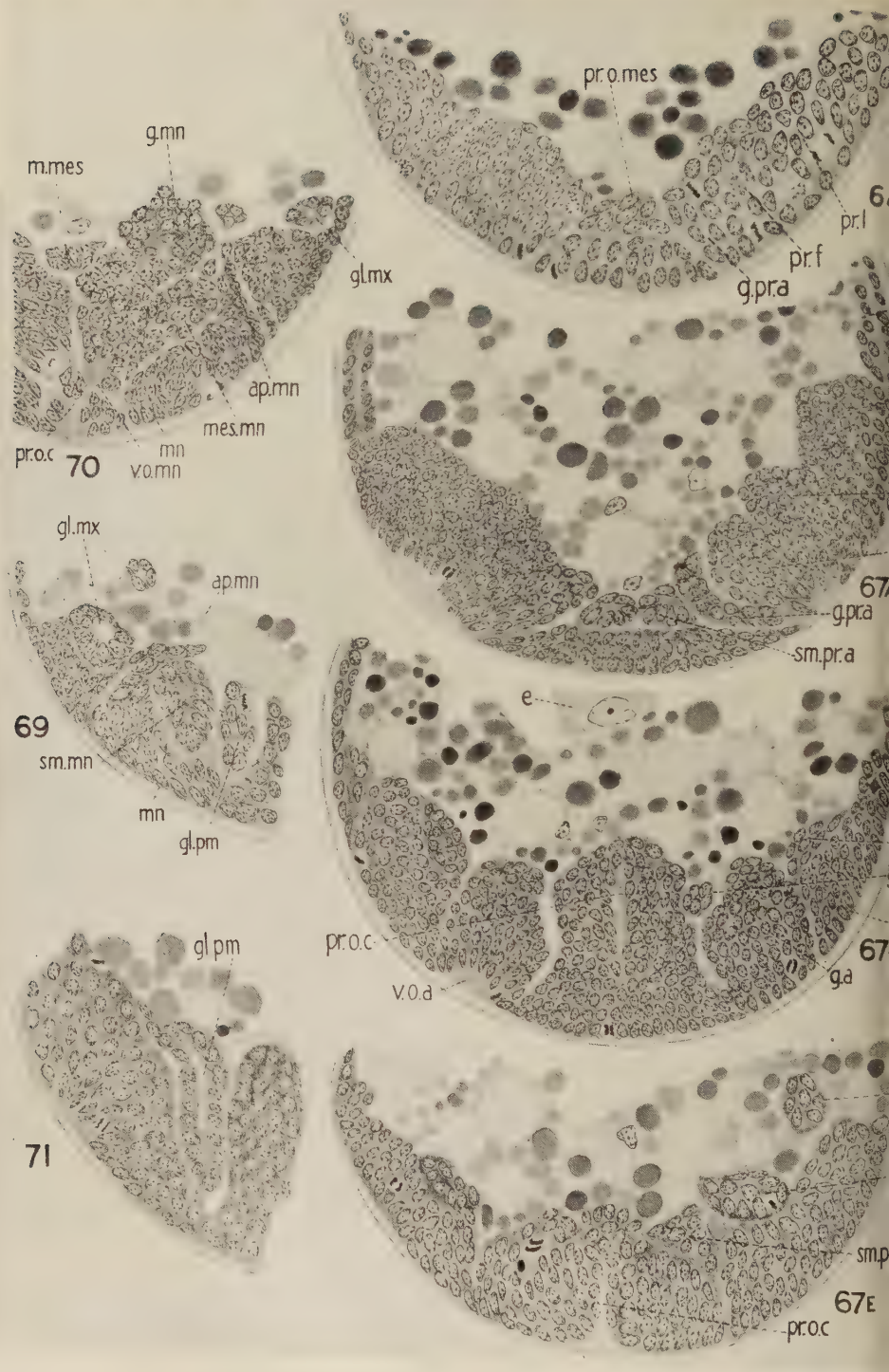
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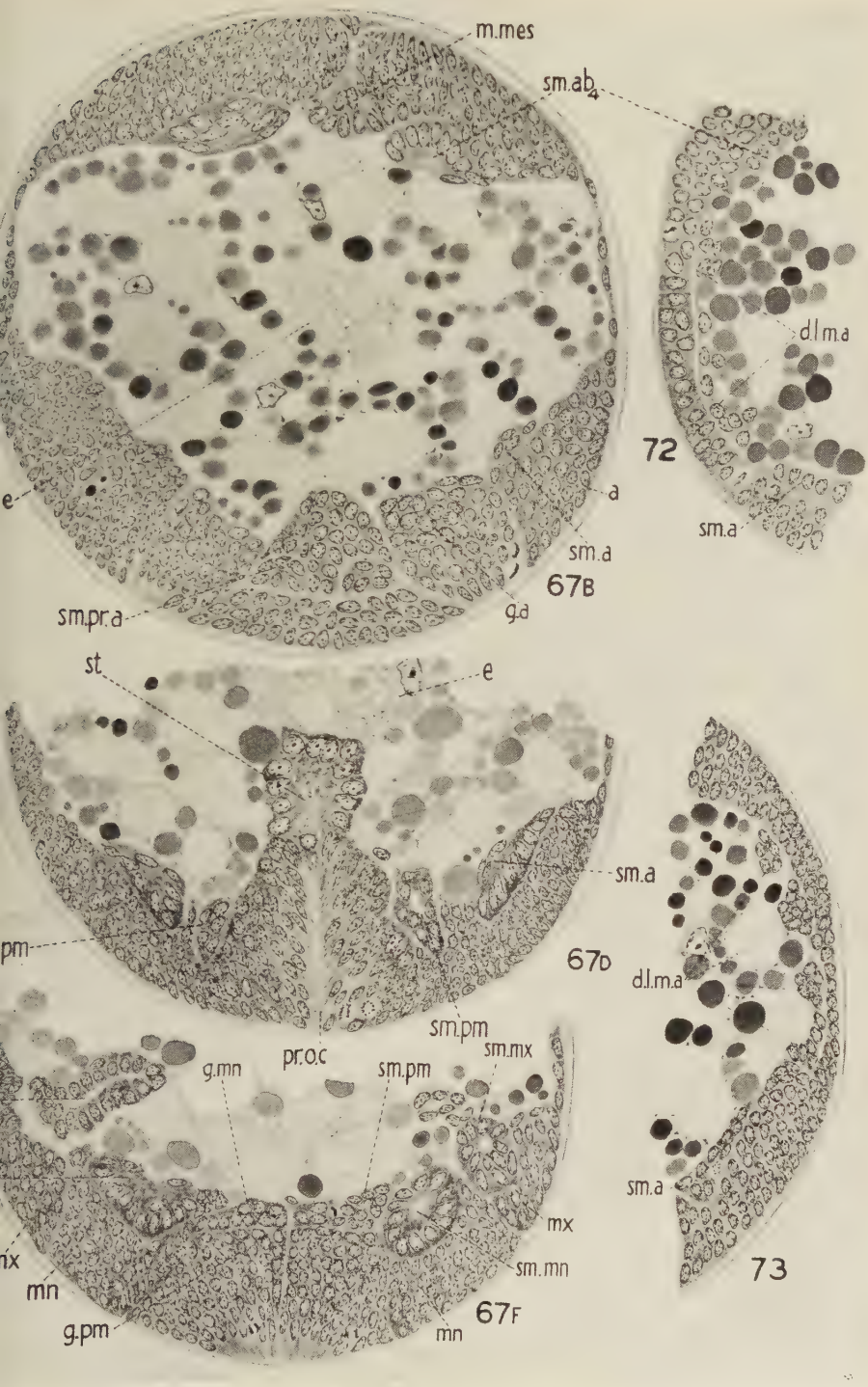
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the height of their development, and also show mitosis. Neuropilem has begun to form in the ganglion. Note the inclusion of 'median mesoderm' in the ganglion. × 950.

Fig. 79. Portion of a section of a 7-day embryo, cut approximately, but not accurately, in the sagittal plane (the embryo is at about the same stage of development as that shown in Text-fig. 5). The section grazes along the lateral wall of the stomodaeum, and shows in succession the pre-mandibular, mandibular, maxillary, collum, and second abdominal somites. × 950.

Fig. 80. Similar section; anterior end to left. The section is from an 8-day embryo. Note that the maxillary somite has begun to elongate to form the salivary gland. The full length of the stomodaeum, which in this embryo has reached the central endoderm, is shown (from an adjacent section) by dotted lines. × 950.

Fig. 81. Similar section of a rather later embryo, anterior end to right. Stomodaeum not present in section. The section shows a more complete set of abdominal somites than does Fig. 80. They are now fully developed, and show each a minute coelomic cavity. Such a cavity is present also in the mandibular somite, but not in that of the collum segment. From the lower end of the maxillary gland a large clump of cells, the future maxillary myoblasts, is in process of separating away. × 950.

Fig. 82. Hinder end of a longitudinal section of a 9-day embryo, showing somites of the anal segment and fifth and fourth abdominal segments. × 950.

Fig. 83. Transverse section through an 8-day embryo, taken a little in front of the proctodaeal opening, the proctodaeum being therefore itself included in the section. In the large accumulation of pre-anal mesoderm no differentiation into somites has yet begun. × 950.

Fig. 84. Similar section, from a late 8-day embryo, showing initial phase in development of fifth abdominal somites. The single primordial germ-cell has now appeared. × 950.

Fig. 85. Similar section, from a 9-day embryo. On the left side a fully formed fifth abdominal somite is now present. × 950.

Fig. 86. Similar section, from an advanced embryo, showing disruption of fifth abdominal somite. The third pair of legs intrude, from in front, into the section. The 'ventral organs' of the fifth abdominal ganglion have appeared. × 950.

Fig. 87. Section through hinder end of a pupa, showing rudiment of the teloblastic mesoderm, from which the mesoderm of the new larval segments will develop. × 950.

Fig. 88. Section similar to that shown in Fig. 64, from an 8-day embryo. The maxillary somite is in process of conversion into the salivary gland. × 950.

Fig. 89. Portion of a section through the collum segment of a 10-day embryo. The mid-gut is in process of formation; on its roof the endodermal cells, with mesodermal investment, are beginning to form a regular epithelium, but its incomplete floor is still composed of large, irregular, and very reticulate 'yolk-cells'. The remainder of the 'yolk-cells' constitute the developing fat-body. The hinder end of the protocerebral ganglion intrudes into the section. The collum ganglion is still associated with its 'ventral organ'. Neuropilem is appearing; note the development of commissural fibres above the 'median mesoderm'. Part of the maxillary gland, including its hinder tip, intrudes into the section; note the developing 'end-sac'. The clump of myoblasts that has arisen by disruption of the collum somite is seen to the right of the ganglion. The two small epidermal cell-aggregations to the sides of the 'ventral organ' are tendon cells for attachment of some dorso-ventral muscles. × 950.

Fig. 90. Section through roof of pre-oral cavity, showing, on left, the earliest detected stage in formation of the clypeal gland. On the right side the gland has not yet begun to form. × 950.

Fig. 91. Portion of a section which grazes along the floor of the clypeus; 10-day embryo. The section actually cuts, below, a little into the pre-oral cavity. Note the developing clypeal glands, growing backward toward the frontal ganglion. × 950.

Plate 8

Fig. 92. Left half of a 'horizontal' section taken at level of pre-oral cavity, from a 9-day embryo. Note that the 'ventral organs' of the developing pre-mandibular segment have moved up to the pre-oral cavity, and are now forming its lateral walls. A fragment of the enlarging pre-mandibular somite intrudes into the section. × 950.

Fig. 93. Part of a section through head of an 8-day embryo (from a 'horizontally' cut series). The section passes immediately in front of the developing pre-oral cavity, and is to be

compared with that shown in Fig. 67 A, which is from a slightly earlier embryo. The small 'ventral organ' of the pre-antennary ganglion is now at its maximum development. A 'ventral organ' cell disposition also involves the whole free surface of the lateral and frontal protocerebral lobes. ×950.

Fig. 94 A–E. Drawing of five approximately 'horizontal' sections through the head of a 10-day embryo. In Fig. B the entire section through the embryo has been drawn, to show (above) the transected abdomen. The series represents an incomplete succession of sections, two having been omitted between B and C, and one between D and E. The series should be compared with Fig. 67 A–F. ×950.

In A are seen the posterior lobes of the protocerebral ganglion, which have grown down from the roof of the head, and have almost met in the mid-line. To the side of these are the lateral lobes of the protocerebrum, of which that on the right side shows 'ventral organ' cell disposition. Anterior to these are the frontal lobes, which have fused in the mid-line; the left shows a 'ventral organ'. On the right side is seen the pre-antennary ganglion. Neuropilem is forming between the three component lobes of the protocerebrum.

B. This section lies immediately below the former. It is a little dorsal to the oesophagus, grazing, at one place, along a fragment of its roof. The lowest part of the posterior protocerebral lobe is present in the section. The deutocerebrum, with neuropilem, is forming, by fusion of the antennary ganglia above the oesophagus. The deutocerebrum therefore lies just below the pre-antennary ganglia, of which a fragment still intrudes from above into the section (right side). The upper half of the section shows the transected abdomen, at the level of the single primordial germ-cell. Note developing trichobothrium.

C. This section passes along the floor of the pre-oral cavity, the latter being transected at one point. The section includes the developing tritocerebrum; note its inferior commissure passing below the oesophagus; note also its 'ventral organs' which are becoming withdrawn from the postero-lateral wall of the pre-oral cavity.

D. This section lies immediately below C, and is taken at the level of the mandibular segment. Note the mandibular ganglion, with its 'ventral organs' forming the floor of the pre-oral cavity. On the right side is seen the developing right hypopharyngeal apophysis, curving back round the ganglion. On both sides may be seen the deep ingrowths of the bases of the mandibles (mandibular apodemes) into the head.

E. This section lies a little distance below D (it should be compared with Fig. 98, which is from a rather earlier embryo). The section passes 'horizontally' along the floor of the maxillary segment, and shows the Anlage of the intermaxillary gland, now separated from the maxillary ganglion. The maxillary 'ventral organ' forms the tip of the intermaxillary plate.

Fig. 95. Section through the collum ganglion of a 10-day embryo, showing its 'ventral organs'. ×950.

Fig. 96. Equivalent section, from a more advanced embryo; a portion of the 'ventral organ' has remained within the sternal epidermis, but the cell-orientation has been lost. From this will develop the 'exsertile vesicle'. Although from a later embryo than the foregoing, the neurilemma has not yet appeared. ×950.

Fig. 97. Similar section, from a pupa, showing developing exsertile vesicle. ×950.

Fig. 98. Section through floor of maxillary segment of a 10-day embryo, to show Anlagen of intermaxillary glands, and beginning of separation of the latter from the maxillary ganglion. Two differentiating neurilemmal cells are seen above the neuropilem. ×950.

Fig. 99. Part of a section of an advanced embryo, from a sagittal series, the section being taken just to the side of the oesophagus. It shows the tritocerebral (pre-mandibular) ganglion, which lies to the side of the oesophagus, also the mandibular, maxillary, and collum ganglia. Note the developing intermaxillary gland lying below the maxillary ganglion. ×950.

Fig. 100. Fragment of a section through lateral head-wall of an advanced embryo, showing development of pseudocular gland (for orientation of section see Fig. 115 A). ×950.

Fig. 101. Drawing of an oblique section through the head of a 10-day embryo. The section is so directed that it passes, on the right side, through the frontal lobe of the protocerebrum, and on the left through the frontal and lateral lobes. The sagittal plane of the embryo, defined by the oesophagus and the septum (*s*) of epidermal cells, is therefore displaced to the right in the section. The obliquity of the section at once brings out the relation of the pre-antennary ganglion to the frontal lobe of the protocerebrum (right side), and to the antennary ganglion and neuropilem of the brain (left side). The relevant sections for comparison are Figs. 93, 94 A. ×950.

Plate 9

Fig. 102. Frontal section through tip of head of a very advanced embryo, to show developing intermaxillary glands. On the roof of the head the section passes (on left) through rear of base of antenna, and shows the developing basal antennary sense organ. Included in the section are the frontal (visceral) ganglion, and part of the clypeal glands. ×780.

Fig. 103. Approximately sagittal section of a 9-day embryo, rather less developed than that shown in Text-fig. 8. The embryo shows the mid-gut in course of acquiring its mesodermal investment, the mesoderm spreading backward over it from the fore-gut. Four endoderm cells are shown in the section, and there is present also a large degenerate yolk-laden cell within the lumen of the developing mid-gut. The pre-antennary somite has disrupted into a clump of cells occupying the cavity of the clypeus. Behind the fourth abdominal segment the mesoderm has not yet differentiated into somites. The unsegmented median mesoderm is exceptionally clearly seen. The developing ganglia are massive and have begun to merge into a continuous chain. The pre-mandibular ganglion lies mostly to the side of the pre-oral cavity, and is therefore not seen; the mandibular ganglion has been drawn up near the hinder margin of the pre-oral cavity. Three 'ventral organs' are present in the section. The 'dorsal organ' is at the height of its development, and its secretion spreads outwards for a short distance under the blastodermic cuticle. ×780.

Fig. 104 A, B. Photographs of two parasagittal sections of a 9–10-day embryo. The photograph has been taken to show, as objectively as possible, the appearance of the 'ventral organs'. The 'ventral organs' seen in Fig. A are those of the collum and second and third abdominal segments. Fig. B, from the adjacent section, shows the maxillary 'ventral organ', and grazes also through the side of the mandibular 'ventral organ'. Other structures seen in the photograph are the 'median mesoderm', with aligned cells, a transected Malpighian tube, and a fragment of the fifth abdominal somite. Note also mid-gut lumen. ×600.

Photographs by Professor E. J. Hartung.

Fig. 105. Portion of a parasagittal section along floor of germ-band of an 8-day embryo, showing early stage in development of the ganglia. The section should be compared with the rather more advanced one shown in Fig. 103. The ganglion-rudiments are not yet continuous; they comprise, from left to right, the ganglia of the maxillary, collum, and first two leg-bearing segments. The 'ventral organs' of the first two only are present in the section. ×780.

Fig. 106. Part of an approximately transverse section through a 10-day embryo; the section passes (below) through the base of the second leg, and (above) through the tergal wall of the fifth abdominal segment, whose developing trichobothrium may be seen. The anterior tip of the hind-gut is also transected, and one of the Malpighian tubes is seen in process of growing out from it. Note the 'ventral organs' in process of incorporation into the ganglia. In the adjacent mesoderm the Anlage of the ventral longitudinal muscle is distinguishable; note also masses of myoblasts within the leg, while others are spreading up the lateral body-wall. ×780.

Fig. 107. Approximately transverse section through third abdominal segment of an advanced embryo. Fragments of the second pair of legs are present in the section. The dorsal body-wall has formed. There is now a well-developed mid-gut, showing marked contrast between the irregularly reticulate cells on its floor and the more compact epithelium on its roof. In the latter concretions have appeared. The 'yolk-cells' are now recognizable as developing fat-body. The nerve-cord has completely separated from the epidermis, and the 'ventral organ' cell disposition has disappeared. The developing ventral longitudinal muscles are now very conspicuous. On the right side is seen a developing coxal apodeme. On the left is seen, in course of development, one of the large muscles of the base of the leg. ×780.

Fig. 108. Similar section to foregoing, from a pupa. There is no further advance in development of the mid-gut. The fat-body has undergone a further depletion of its yolk. It has begun to shrink from the neighbourhood of the nerve-cord, thereby revealing the epineural and lateral neural blood-spaces. The ventral longitudinal muscles have moved into their definitive positions to the side of the nerve-cord. ×780.

Fig. 109. Similar section, from a very young first instar larva. The development of the mid-gut wall is now complete, and it is wholly enclosed by mesoderm. A 'striated border' has formed in the mid-gut epithelium, but is absent on the floor-cells. The fat-body is completely denuded of its yolk, and reserve products have not yet begun to accumulate, the blood-spaces being therefore wide. ×780.

Fig. 110. Frontal section through head of a 9-day embryo. The section passes along the length of the antenna, 'horizontally' through the floor of the mandibular segment, and grazes

the maxillary segment below. The antenna is occupied by a clump of cells, derived from the disrupting antennary somite; some of its cells are extending back, under the roof of the head, to form the tergal muscle of the antenna. Median to the antenna is the antennary ganglion (developing deutocerebrum), and below it a fragment of pre-mandibular ganglion (developing tritocerebrum). The ectoderm along the lateral margins of the mandible and maxilla is growing into the head to form their respective apodemes, which are drawing in some of the mesoderm with them. ×780.

Fig. 111. Frontal section through right half of head of an embryo aged about 10 days. The plane of section is so directed that the pre-mandibular gland is included for its entire length in the section. Below it lies the mandible, now deeply ingrown into the cavity of the head, and displaying at its inner end the developing lateral mandibular ligament. Below the mandible the section grazes the surface of the maxilla. In the epidermis some unusually large cells are to be seen; those above the brain are setigerous cells, those to the right of the brain are the enlarging cells of the pseudocular gland. In the brain we can distinguish the two diminutive pre-antennary ganglia, between which the septum of ectodermal cells is growing down from above. To the side of the pre-antennary ganglion, the great mass of ganglionic tissue is the protocerebrum, of which the frontal and lateral lobes are distinguishable. Below the pre-antennary ganglion is seen a portion of the developing deutocerebrum, the antennary ganglia having begun to fuse above the oesophagus. A portion of the deutocerebrum also intervenes between the protocerebrum and the pre-mandibular ganglion (developing tritocerebrum), which may be seen to the side of the oesophagus. ×780.

Fig. 112. Portion of a frontal section through an advanced embryo, showing the cells of the hypopharyngeal apophysis becoming connected with the overlying antennary and the underlying mandibular mesoderm. For an earlier stage of development, see Fig. 115 A.

Fig. 113. 'Horizontal' section along hind-gut of an advanced embryo, showing incipient differentiation of colon and rectum. Note the incorporation of some hind-gut cells into the wall of the mid-gut. The Malpighian tubes are beginning to develop. ×780.

Fig. 114. 'Horizontal' section of mid-gut of a young pupa; the ends of the oesophagus and hind-gut are included in the section, and, on the right side, a Malpighian tube (base of other Malpighian tube on left). Differentiation of the Malpighian tube into its three regions has already begun. Note excretory concretions in mid-gut cells. ×780.

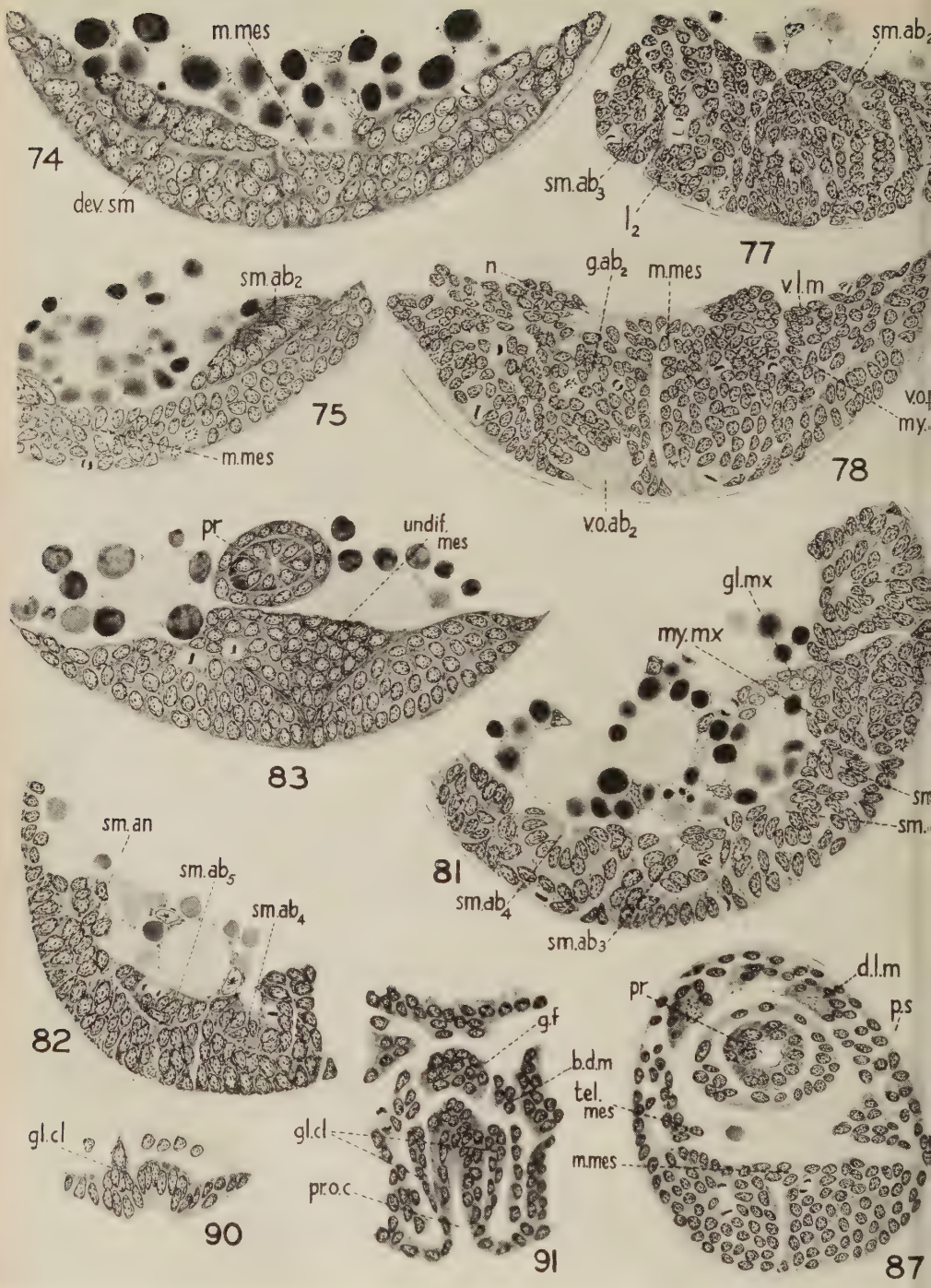
Fig. 115 A, B. A is a drawing of a frontal section through the anterior end of a 10-day embryo. The section passes (above) through the protocerebrum, grazes along the hinder surface of the deutocerebrum and tritocerebrum, and transects (below) the 'ventral organs' of the maxillary segment. The ganglion above the 'ventral organ' is not purely maxillary ganglion: it is the sub-oesophageal ganglion, of which the lower part in contact with the 'ventral organs' is of maxillary, the upper of mandibular, origin. The mandibular ventral organs, of course, lie anterior to the plane of section (for orientation of section refer to Text-fig. 8). To the sides of the sub-oesophageal ganglion are seen the transected inner ends of the mandibular apodemes, from which a few cells are beginning to grow medially across the mandibular ganglion; they give the first indication of the oesophageal (visceral) ganglia. The small clump of cells between the mandibular apodeme and the fragment of tritocerebrum is the hindermost tip of the hypopharyngeal apophysis. A little antennary mesoderm intrudes from in front into the section, a fragment of the antenna being also present. In the lowest part of the section is seen a little maxillary mesoderm. To the side of the protocerebrum the development of the pseudocular gland is in progress.

B is a fragment of the immediately succeeding section of the same embryo. The section shows the maxillary apodeme and the mesoderm of the segment, growing up the side of the ganglion, towards the hypopharyngeal apophysis, whose hinder end is seen in the preceding section. ×780.

The following abbreviations are used in the lettering of the Plates:

a antenna; *a.o* anal opening; *ap.cx* coxal apodeme; *a.pl* anal plate; *ap.mn* mandibular apodeme; *ap.mx* maxillary apodeme; *b.a.s.o* basal antennal sense organ; *b.c* blastodermic cuticle; *b.d.m* buccal dilator muscle; *br* brain; *c* colon; *cl.f* clypeal fold; *c.pr* protocerebral commissure; *c.tr.i* inferior tritocerebral commissure; *dev.sm* developing somite; *d.l.m* dorsal longitudinal muscle; *d.l.m.a* Anlage of dorsal longitudinal muscle; *d.mx* exit duct of maxillary gland; *d.o* 'dorsal organ'; *d.pm* exit duct of pre-mandibular gland; *dt* deutocerebrum; *d.y.c* degenerating yolk-cells; *e* endoderm; *e.d* developing exit duct of reproductive organs; *e.d.a* anterior part of ejaculatory duct; *e.d.deg* degenerating exit duct of female reproductive system; *e.d.g* glandular

part of ejaculatory duct; *ep* epidermal cells; *ep.s* epineural sinus; *e.s* 'end sac' of salivary gland; *ex.v* exsertile vesicle (?); *f.b* fat-body; *f.b.s* developing secondary fat-body; *f.c* follicle cell; *f.p.n* female pro-nucleus; *g.a* antennary ganglion; $g.ab_{2,3}$, &c. ganglia of second, third, &c. abdominal segments; *g.c* ganglion of collum segment; *g.f* frontal (visceral) ganglion; *gl* globulus; *gl.cl* clypeal gland; *gl.i.mx* intermaxillary gland; *gl.mx* maxillary (salivary) gland; *gl.pm* pre-mandibular gland; *gl.ps* pseudocular gland; *gm* germarium; *gm.c* germ-cells; *g.mn* mandibular ganglion; *g.mx* maxillary ganglion; *gn.a* genital atrium; *gn.t* genital tube (as distinct from its content of germ-cells); *gn.t.a* Anlage of genital tube; *g.oes* oesophageal ganglion (?); *g.oes.a* its Anlage; *g.pm* pre-mandibular ganglion; *g.pr.a* pre-antennary ganglion; *g.sb.oes* sub-oesophageal ganglion; *g.tel* teloblastic ganglion; *h.a* hypopharyngeal apophysis; *h.a.a* ascending arm of latter; *h.g* hind-gut; *h.l* head-lobe; *i.mx.pl* intermaxillary plate; $l_{1,2}$, &c. first, second, &c. legs; l_5a Anlage of fifth leg; *l.m.h* levator muscle of head; *l.n.s* lateral neural sinus; *mes* mesoderm; *mes.mn* mandibular mesoderm; *mes.mx* maxillary mesoderm; *mes.s.a* sternal mesoderm of antenna; *m.g* mid-gut; *m.g.mes* mid-gut mesoderm; *m.g.p* proctodaeal component of mid-gut; *m.mes* 'median mesoderm'; *mn* mandible; *mn.l.l* lateral 'ligament' of mandible; *mn.l.m* median ligament of mandible; *mn.st* mandibular sternite; *m.p.n* male pronucleus; *m.t* Malpighian tube; *m.terg.a* tergal muscle of antenna (Anlage of); *mx* maxilla; $my.ab_{2,3}$, &c. myoblasts of second, third, &c., abdominal segments; *my.c* myoblasts of collum segment; *my.mx* myoblasts of maxillary segment; *n* neuropilem; *ng* neuroglia cells; *nl* neurilemma; *o* stomodaeal opening; *oc* oocyte; *od* oviduct; *oes* oesophagus; *oes.d.m* oesophageal dilator muscle; *og* oogonia; *o.s* occipital suture; *ov* ovary; *o.w* ovarian wall; *p* penis; *p.b* polar body; *pr* proctodaeum; *pr.f* frontal lobe of protocerebrum; *pr.g.c* primordial germ-cell; *pr.l* lateral lobe of protocerebrum; *pr.mes* proctodaeal mesoderm; *pr.o.c* pre-oral cavity; *pr.o.mes* pre-oral mesoderm; *pr.p* posterior lobe of protocerebral ganglion; *prt* protocerebrum; *p.s* pupal sheath; *r* rectum; *r.s* receptaculum seminis; *r.s.a* Anlage of latter; *r.v* rectal valve; *s* septum of epidermal cells growing down between pre-antennary ganglia; *s.a* anal segment; $s.ab_{2,3}$, &c. second, third, &c., abdominal segments; *s.c* collum segment; *sm.a* antennary somite; $sm.ab_{2,3}$, &c. second, third, &c., abdominal somites; *sm.an* anal somite; *sm.c* somite of collum segment; *sm.mn* mandibular somite; *sm.mx* maxillary somite; *sm.pm* pre-mandibular somite; *sm.pr.a* pre-antennary somite; *s.pm* pre-mandibular segment; $s.s_{2,3}$ second, third sensory setae (trichobothria); *s.s.a* Anlage of sensory seta (trichobothrium); the particular seta may be identified by a numeral, e.g. $s.s._2a$, &c.; *st* stomodaeum; *st.c* setigerous cell; *st.m* musculature of stomodaeum; *st.mes* stomodaeal mesoderm; *sus* suspensorium of hypopharyngeal apophysis; *t* testis; *tel.mes* teloblastic mesoderm; *tn* 'tendon' cells, for attachment of muscles; *tr* tritocerebrum; *undif.mes* undifferentiated mesoderm; *v.d* vas deferens; *v.l.m* ventral longitudinal muscle (segmental allocation of this muscle sometimes indicated by numerals); *v.l.m.a* its Anlage (segmental allocation sometimes indicated by numeral); *v.o.a*, $v.o.ab_{2,3}$, &c., *v.o.c*, *v.o.mn*, *v.o.m.x*, *v.o.pm* 'ventral organs' of antennary, second and third, &c., abdominal, collum, mandibular, maxillary, and pre-mandibular segments respectively; *v.o.tel* 'ventral organ' associated with teloblastic ganglion; *v.s* vesicula seminalis; *v.s.a* its Anlage; *x*, *y* for various uses of these letters see legend to figures in which they appear; *y.c* yolk-cells; *y.n* yolk nucleus; *z* as for *x*.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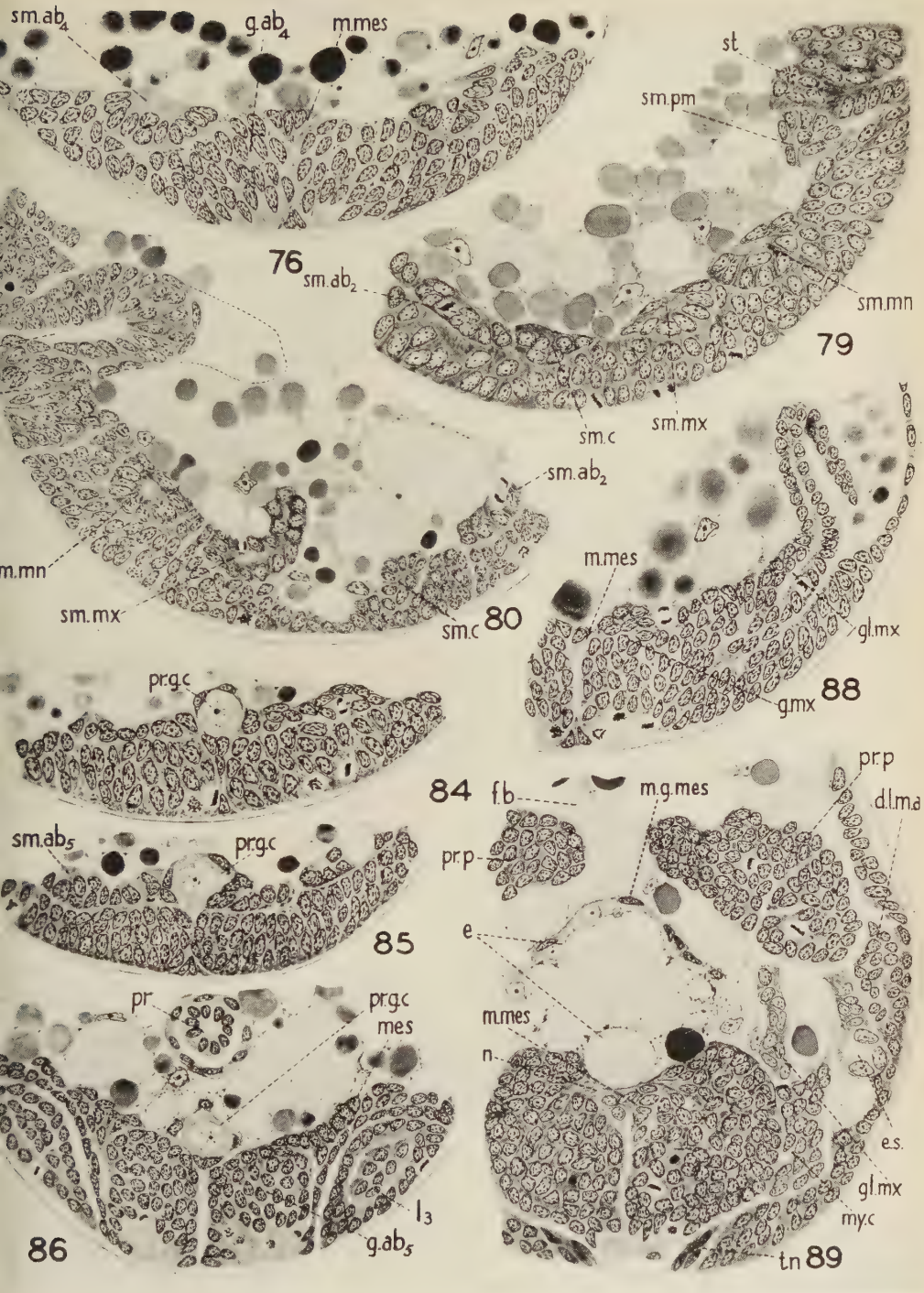
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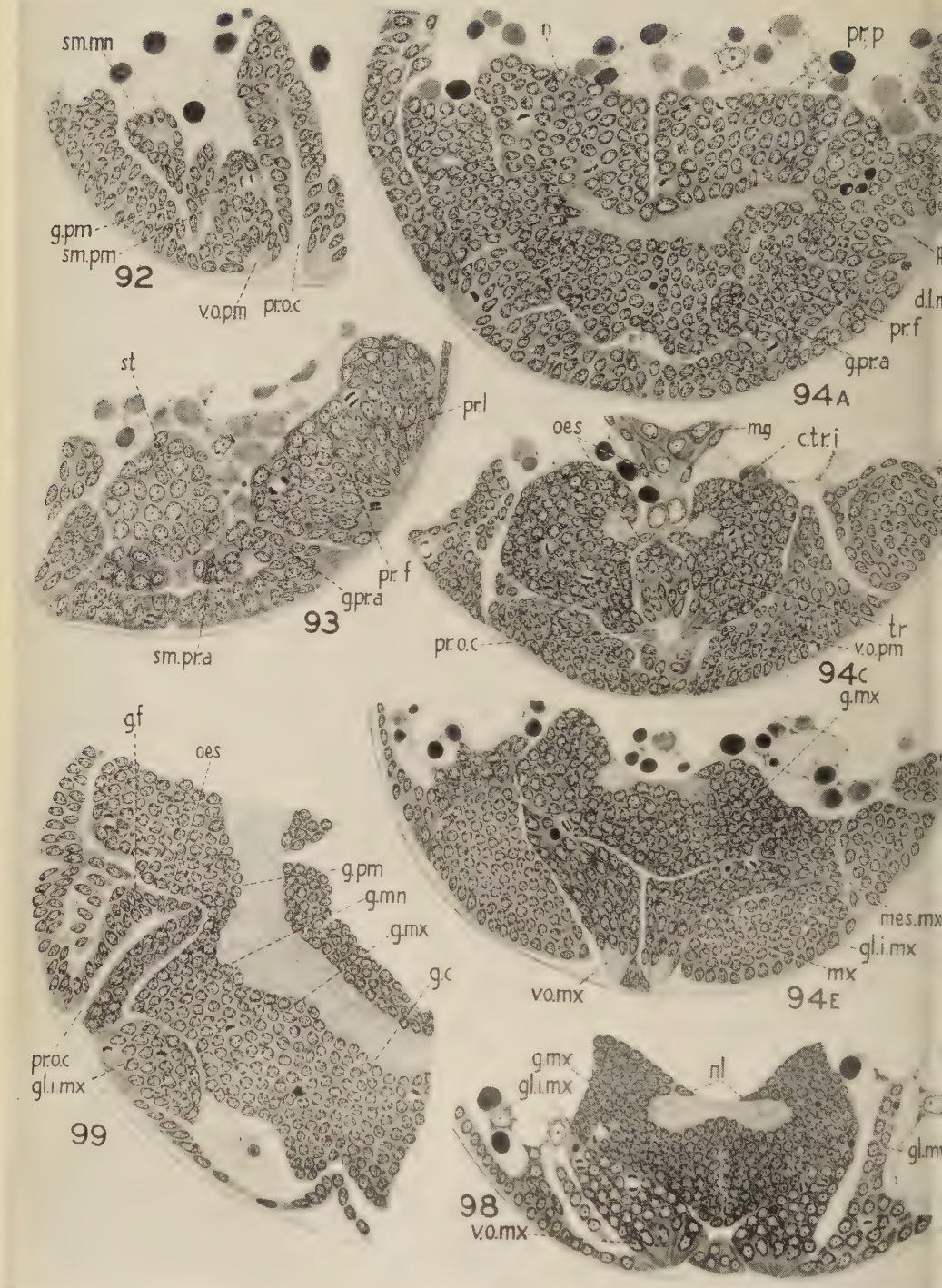
PLATE VII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94C
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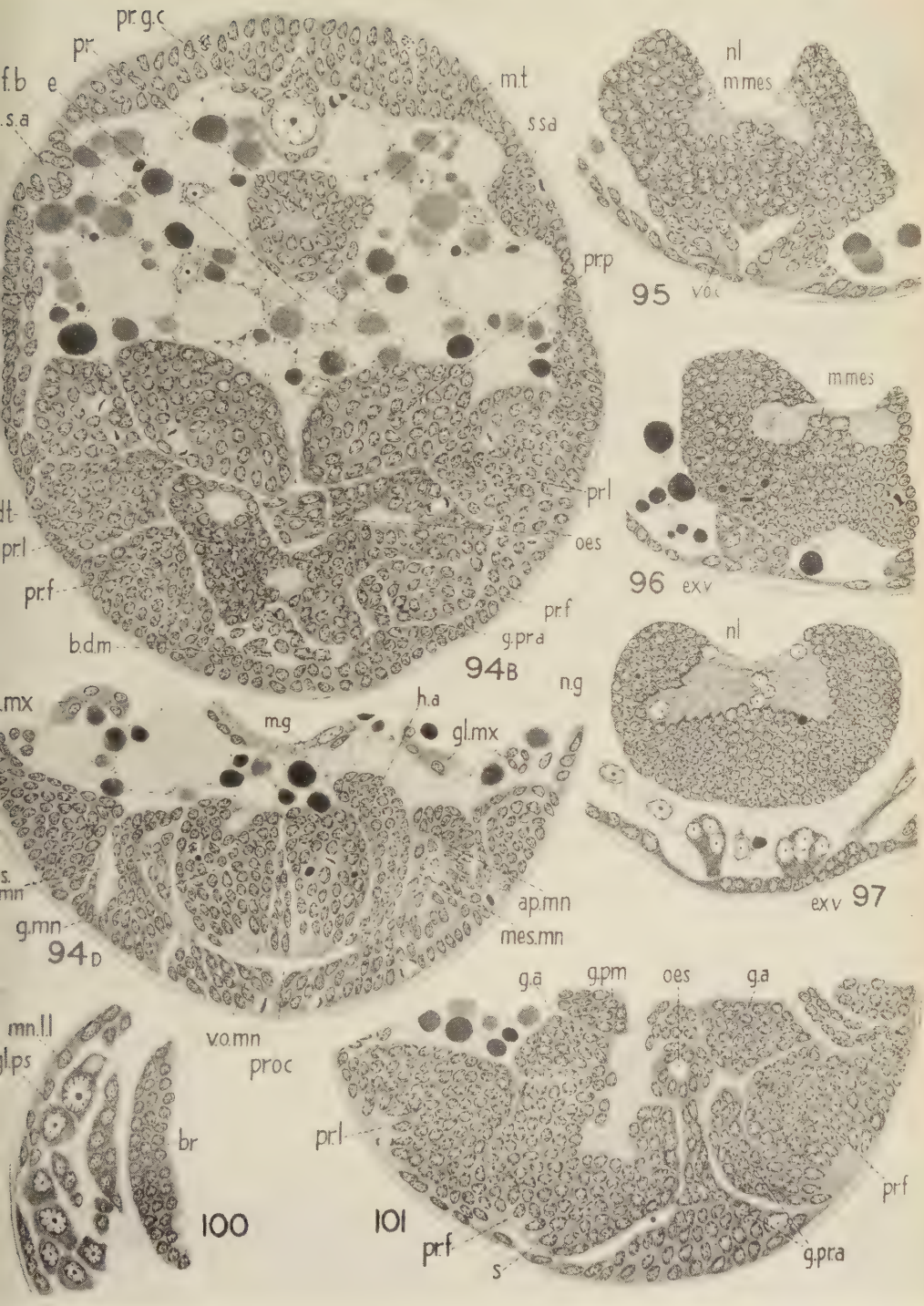
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PLATE VIII

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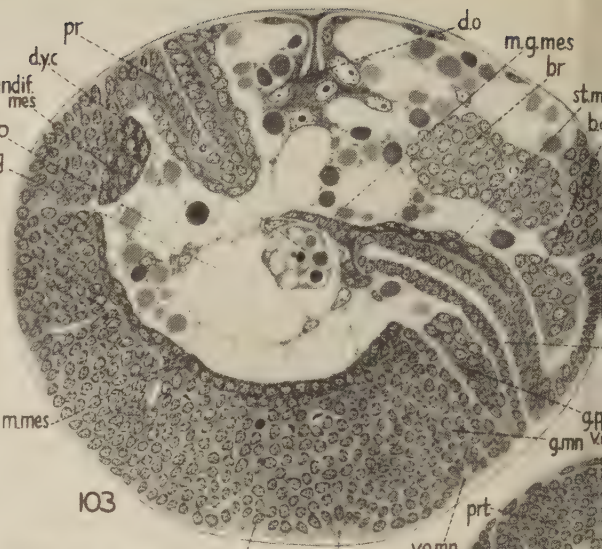

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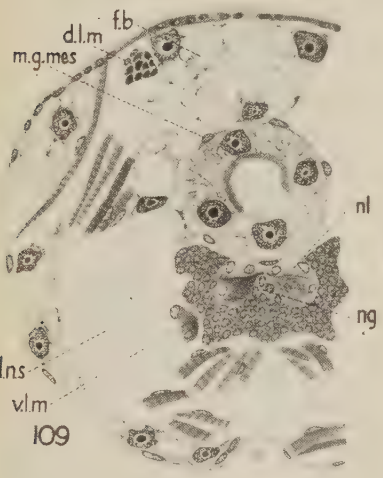

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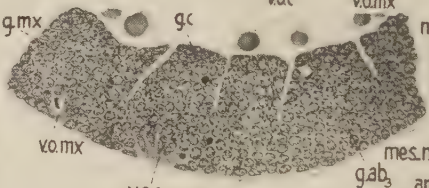

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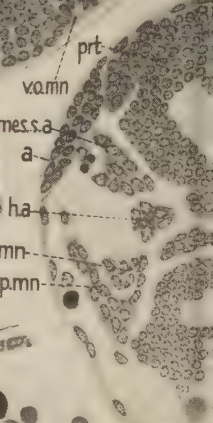

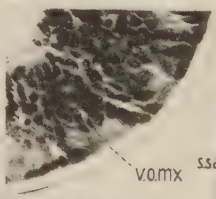

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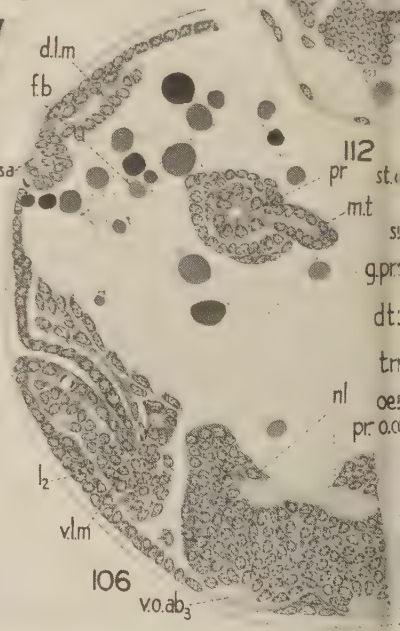

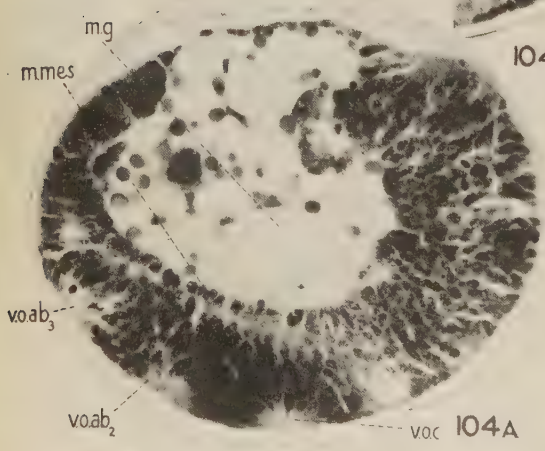

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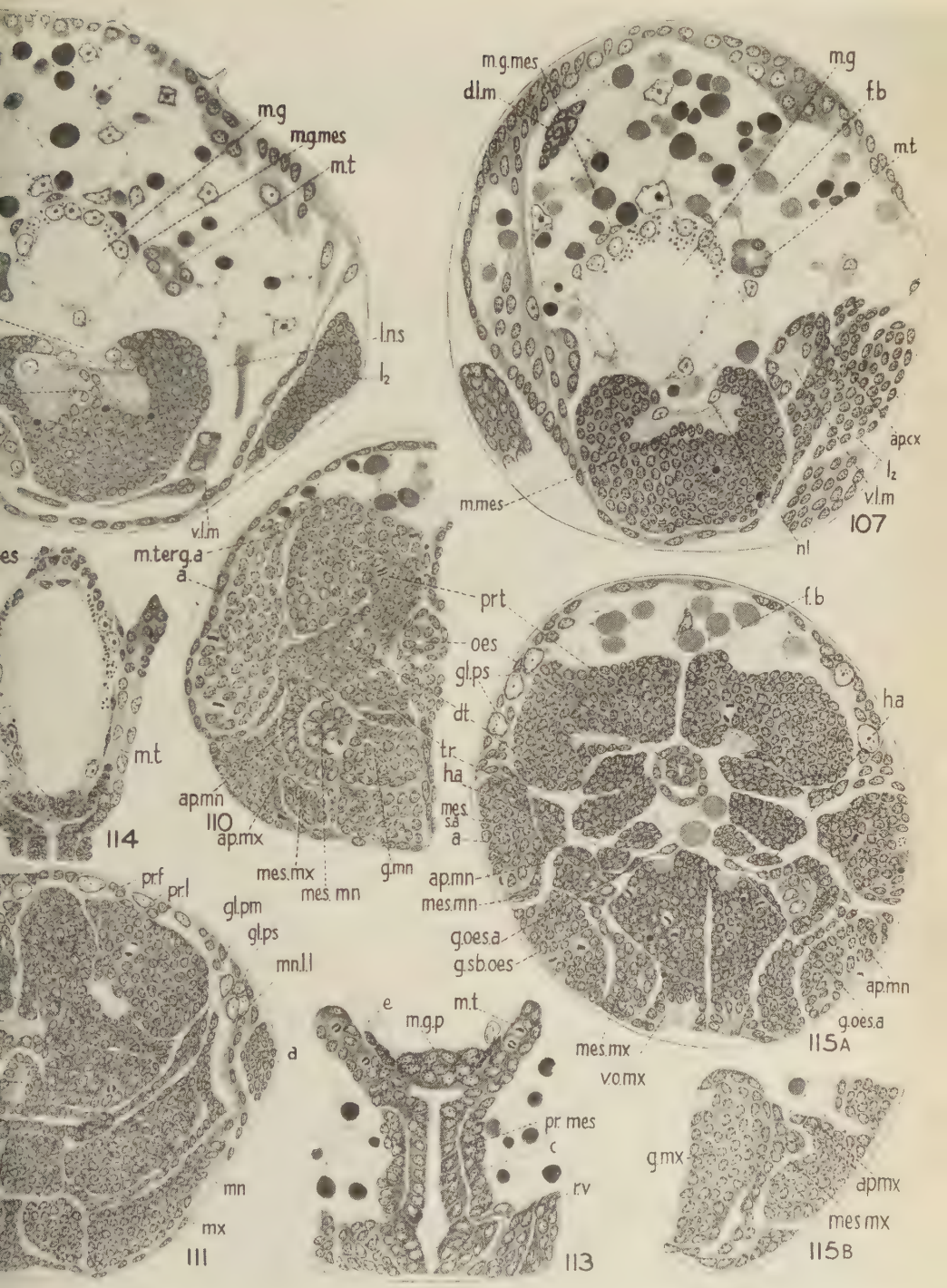
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PLATE IX

Some Observations with the Phase-contrast Microscope on the Neurones of *Helix aspersa*

BY

OWEN LEWIS THOMAS, M.D. (N.Z.)

Beit Memorial Medical Research Fellow

(*From the Department of Zoology and Comparative Anatomy, Oxford*)

With 1 Text-figure

ALTHOUGH various investigations have contributed to the development of phase-contrast microscopy from the time of Abbe, the production of a completed microscope available for use in biological and allied fields has been a comparatively recent achievement (Köhler and Loos, 1941). A good review of this promising advance is given by Bennett (1946).

The neurones of *Helix aspersa* have been chosen for the present study as these cells are large and easily isolated in a fresh state, as well as for their having already been the subject of a number of cytological investigations (Kolatchev, 1916; Brambell, 1923; Brambell and Gatenby, 1923; Boyle, 1937). The following observations are limited to a description of the appearance of the neurofibrils in these cells together with a description of some very small particles shown to be present in their protoplasm, distinct from and of a much smaller order than the mitochondria. A further communication is on hand concerning some observations on the other inclusions and organoids of these cells.

Technique

The large motor neurones of the post-cerebrum of *Helix* were dissected, with the aid of a Greenough microscope, in a few drops of 0·7 per cent. sodium chloride containing 0·2 per cent. of 10 per cent. anhydrous calcium chloride. The fluffy nerve-cell mass can be detached from the firm connective tissue capsule of the cerebral ganglion and subsequently teased and flattened in a drop of the indifferent solution between cover-glass and slide. In this way it is possible to display a single-cell layer of the large neurones arranged in a fan-shaped manner, their unipolar cell bodies and stout axons resembling a group of captive toy balloons. The degree of flattening of the cells due to the cover-glass pressure makes possible high-power observations on their cytoplasm with the phase-contrast microscope. Similar preparations examined in hanging drops were found to be suitable only for low- and medium-power observation, as the full benefit of phase-contrast illumination can apparently only be obtained in preparations consisting of a single layer of cells.

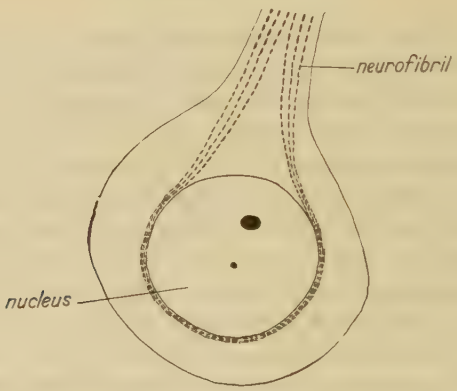
The microscope used was kindly lent me by Dr. W. Loos and was supplied with an annular illuminant, pankratic condenser, and an annular diffraction

plate of the A+ type of Bennett (1946) incorporated within the optical system of an achromatic 1·25 n.a. homogeneous immersion lens. This was used in conjunction with a 10× compensating ocular.

Observations

(*a*) *The Neurofibrils*

The large unipolar neurones exhibit a groundwork of very fine filaments which are arranged in rows parallel to the axon. Each filament is delicately beaded in structure and resembles a very long chain of minute cocci. As the axon hillock is reached the rows of fibrils fan out and form a continuous



Text-fig. 1. Schematic drawing of the arrangement of the neurofibrils in a large neurone of *Helix* as seen in optical section with the phase-contrast microscope.

filamentous sheath enveloping the nucleus, the individual fibrils passing in an uninterrupted fashion around the nucleus and back into the axon. No evidence of lateral anastomoses of these filaments either within the axon or in the cell body was observed.

With ordinary transmitted illumination they cannot with certainty be recognized; but if a quick transition from transmitted to phase-contrast illumination be made on the same field, the impression of a very faint striation at once gives place to the well-defined picture described above. This experiment is itself a very striking one and shows to full advantage the superiority of the new method of illumination for the study of these structures.

As a 'control' to these observations *in vivo*, preparations were made with the classical reduced silver technique of Cajal as modified by Nonidez (1939) and the presence of neurofibrils in *Helix* neurones was established by this method. In this way their size and general distribution within the cell conforms to the appearances seen with the phase-contrast microscope, but the beaded appearance of the fresh neurofibrils was not reproduced in the fixed and silvered tissue. Moreover, their regular and orderly arrangement in the fresh cell was severely distorted by the fixation and silvering process. This was especially noticeable about the nucleus, where the neurofibrils appear to form a coarse network of anastomosing threads.

(*b*) *The Microneurosomes*

When preparations of the teased cells compressed between cover-glass and slide are watched for a few minutes, spherical blebs of cytoplasm are seen to exude from the cell membrane of the neurones and finally to detach themselves and float away in the surrounding medium. These droplets usually appear as clear homogeneous filtrates of the cell cytoplasm when ordinary transmitted illumination is employed in their examination. Occasionally a few cell granules such as mitochondria may escape into the droplet from the parent cell.

If the phase-contrast microscope is turned to their examination the impression of this clear homogeneity is immediately lost and they are found to be teeming with an immense number of very small particles moving rapidly with an intense Brownian motion. The individual particles can be distinctly resolved by the eye but it is impossible to see their exact shape and it is impossible to measure them by any of the means ordinarily at the disposal of the microscopist.

After a few minutes of very active movement the tiny particles slowly commence to agglutinate and finally the whole droplet becomes a motionless pale-grey mass of cytoplasm. It was found that 10 per cent. formol or 70 per cent. alcohol run under the cover-glass by diffusion produced an instantaneous fixation and agglutination of the particles. Furthermore they are conveniently made to adhere either to the slide or cover-glass and the preparation can then be stained and mounted in balsam. Toluidine blue and methylene blue were both used as staining agents and strongly stain the agglutinate.

Attempts were made to stain these particles with vital dyes, and neutral red 1 : 10,000 in saline was found to give the best results. In this way the tiny particles are quickly tinged a brick-red colour and although their small size precludes accurate description as to shape, the individual particles are distinctly coloured. Similarly the agglutinated mass retains the red colour. When stained vitally the particles are visible with transmitted light provided a well-corrected immersion lens be used with a wide cone and 3 mm. diaphragmed source of light. These particles are not to be confused with the vacuome of Parat, which in these cells consists of much larger red-staining spheres.

It must be stressed that these particles are only seen to perfection once they have been expressed from the cell. With careful searching similar particles can be seen to occur within the intact cell, especially over the nucleus where the cytoplasmic layer is naturally reduced in thickness. Boyle (1938) states that he never witnessed Brownian movement within *Helix* neurones, using this argument to support his contention that the cytoplasm of the cell in life is of semi-solid consistency. I am not in agreement with this finding.

Discussion

The status of the neurofibrils as structural components of the living nerve-cell has long been a bone of contention among neurologists. Since their first reported discovery by Remak (1843) in the freshly teased nerve-cells of the

crayfish, all manner of opinion has been expressed from time to time concerning the reality of what Cajal has called the 'enigmatic warp' (Marinesco, 1911; Mott, 1912; Bozler, 1927; de Renyi, 1932). Claims for their existence in the neurones of a wide variety of both vertebrates and invertebrates have always been answered by counterclaimants who attribute their appearances as artifacts produced by the manipulative procedures of technique.

By virtue of its construction the phase-contrast microscope allows us to visualize very slight variations of optical density within what would appear as an optically homogeneous medium. Attention is drawn in this paper to the fact that structures apparently identical with the classical neurofibrils can be beautifully shown up by applying this principle of microscopy to teased cells lying in indifferent media. The only element of manipulative procedure inflicted upon the cells is the pressure of the cover-glass found necessary to flatten them adequately for high-power observation.

The very small particles appear to be structures peculiar to neurones. A large number of other tissues from the snail as well as from vertebrates have been examined with a similar technique, so far with negative results. In size and general conformity the particles appear to be of the order of phage-particles recently seen with the phase-contrast microscope (Hofer and Richards, 1945).

Although the possibilities of phase-contrast microscopy do not appear to have been fully developed, the instrument in its present form does not effectively increase resolution as such, nor does it allow us to visualize colloid particles in the manner of the ultramicroscope.

I am quite certain that the particles I have described do not correspond to those already observed in vertebrate neurones by Mott (1912) in his studies with the paraboloid dark field condenser and the ultramicroscope. Mott describes his particles as being less than one micron in size and sometimes spherical and sometimes oval in shape. He considered that they consisted of a colloidal fluid surrounded by a membrane possibly consisting of lipoids. Particles answering this description do occur in my material and are quite distinct from the much smaller neutral red-staining granules. As these granules can hardly be classed with the submicroscopic particles of Claude (1943) and further in size and staining reactions can be differentiated from the granular mitochondria of the neurone (the neurosomes of Held), I propose the name 'microneurosomes' for their description.

In conclusion I wish to thank Dr. J. R. Baker for his help and encouragement throughout this study.

Summary

1. Phase-contrast microscopy reveals the presence of neurofibrils within the freshly teased neurones of *Helix aspersa*.

2. Each fibril resembles a row of minute cocci. Individual fibrils appear to pass from the axon to the cell body, loop round the nucleus, and retaining their identity pass back into the axon. No evidence of lateral anastamoses nor network formation of the fibrils could be found.

3. Small granules, staining vitally with neutral red and designated 'micro-neurosomes', have been found in the cytoplasm of the neuron. They are distinct from and smaller than the mitochondria.

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